# Utilization of By-products of Acacia processing for Biogas Production

HAMK
UNIVERSITY OF APPLIED SCIENCES

Bachelor's thesis

Degree Programme in Biotechnology and Food Engineering

HAMK University of Applied Sciences

*Katja Lehkonen*

HAMK
UNIVERSITY OF APPLIED SCIENCES

ABSTRACT

VISAMÄKI
Degree Programme in Biotechnology and Food Engineering
Environmental Biotechnology

**Author** Katja Lehkonen **Year** 2016

**Subject of Bachelor's thesis** Utilization of By-products of Acacia Processing for Biogas Production

ABSTRACT

Acacia is a widely used raw material in South America, South Africa and Australia. Acacia is processed for different purposes e.g.in manufacturing barbeque char and tannin extracts which are further refined as biopolymers. By-products generated in processing are poorly utilized. Processing residues are mostly landfilled instead of considering them as a value added raw material. Thus, utilization of these by-products is an important new research subject due to increasing use of acacia tree worldwide.

The aim of this Bachelor's thesis was to study the utilization of several by-products generated in the production processes of tannin extracts and charcoal and their potential use as biogas. Another aim was to examine the influence of different pre-treatments to enhance methane production of acacia by-product samples. The samples studied were extraction residue and sludge from tannin extract process and pyrolysis gas condensate (pyroligneous liquor) from char production.

Alkaline pre-treatments in various conditions and bio methane potential test (BMP) were conducted in the thesis. Alkaline pre-treatments are effective in altering the structure of lignin. The challenge in using lignocellulosic material for biogas production is their structure and composition. Alkaline pre-treatment was chosen to treat lignocellulosic samples prior to the methane potential (BMP) test. Pre-treatments were conducted with sodium hydroxide (NaOH), calcium oxide (CaO) and calcium hydroxide ($Ca(OH)_2$). The theoretical biogas potential was also measured with Flash BMP (by near infrared spectroscopy) and by calculating from COD.

The results of the thesis show that the sodium hydroxide treatment was the most effective pre-treatment as it enhanced the biogas production by 40-80%. The temperature also had an effect. The treatment of extraction residue with a high temperature enhanced the gas production by almost 80 %. It can be concluded that alkaline pre-treatment enhanced biogas production with extraction residue, but not with sludge. Bark and pyroligneous liquor are not suitable for biogas production without any treatments.

**Keywords** Anaerobic digestion, methane potential, pre-treatment, acacia mearnsii

**Pages** 36 p. + appendices 10 p.

HAMK
HÄMEEN AMMATTIKORKEAKOULU

VISAMÄKI
Bio- ja elintarviketekniikan koulutusohjelma
Ympäristöbiotekniikka

TIIVISTELMÄ

**Tekijä** Katja Lehkonen **Vuosi** 2016

**Työn nimi** Akasian prosessoinnissa syntyvien sivujakeiden hyödyntäminen biokaasun tuotannossa

TIIVISTELMÄ

Akasiaa käytetään merkittävästi raaka-aineena Etelä-Amerikassa, Etelä-Afrikassa sekä Australiassa. Sitä hyödynnetään erilaisissa käyttökohteissa, kuten grillihiilien ja tanniiniuutteiden valmistuksessa. Tuotanto aiheuttaa erilaisia sivutuotteita, jotka ovat vielä huonosti hyödynnettyjä ja pääosin sijoitettu kaatopaikalle. Akasian käytön kasvaessa maailmanlaajuisesti, näiden sivutuotteiden hyödyntäminen on tärkeä tutkimuskohde.

Tässä työssä keskitytään akasiasta tuotettujen tanniiniuutteiden ja puuhiilen valmistuksessa syntyvien sivujakeiden hyödyntämiseen ja niiden biokaasupotentiaalin selvittämiseen. Sivujakeita tutkittiin kokeellisesti biokaasun tuottotestillä sellaisenaan ja eri tavoin esikäsiteltyinä. Tarkoituksena oli selvittää esikäsittelyiden vaikutusta näytteiden biokaasutuottoon. Tutkittavat näytteet olivat tanniinien valmistuksessa syntynyt uuttojäännös ja liete sekä puuhiilen valmistuksessa syntynyt pyrolyysikondensaatti.

Työssä suoritettiin emäksisiä esikäsittelyjä eri olosuhteissa ja biometaanipotentiaalitesti (BMP). Emäksinä käytettiin natriumhydroksia (NaOH) sekä kalsiumoksidia (CaO) ja kalsiumhydroksidia ($Ca(OH)_2$). Teoreettinen metaanipotentiaali mitattiin Flash BMP:n avulla ja COD:sta laskemalla.

Natriumhydroksidikäsittelyllä saatiin tehostettua biokaasun tuottoa 40-80%. Myös käsittelylämpötilalla oli vaikutuksia metaanin tuottoon, sillä kuumakäsittely tehosti kaasun tuottoa 80 %. Yleisesti voidaan todeta, että esikäsittelyt paransivat biokaasun tuottoa uuttojäännöksen käsittelyssä, mutta ei lietteen käsittelyssä. Käsittelemätön kuori ja pyrolyysikondensaatti eivät sovellu biokaasun tuottoon ilman käsittelyä.

**Avainsanat** Biokaasu, mädätys, metaanipotentiaali, esikäsittely, Acacia mearnsii

**Sivut** 36 s. + liitteet 10 s.

CONTENTS

Appendix 2 Dry weight and solubilisation
Appendix 3 Phenols

Appendix 4/1 BMP results from extraction residue test

Appendix 4/2 BMP graphs from extraction residue test with standard deviation (NaOH-bottles and reference)

Appendix 4/3 BMP graphs from extraction residue test with standard deviation (CaO- and $CaOH_2$-bottles and bark)

Appendix 4/4 BMP results from sludge test

Appendix 4/5 BMP graphs from sludge test with standard deviation (NaOH-bottles and reference)

Appendix 4/6 BMP graphs from sludge test with standard deviation (CaO- and $CaOH_2$-bottles and pyroligneous liquor)

# 1 INTRODUCTION

Acacia is a greatly used raw material in South America, South Africa and Australia. There are several varieties of acacia trees, and this thesis will focus on *Acacia mearnsii*, which is a traditional tannin source. Acacia is processed for different purposes, like production of barbeque char (biochar) and tannin based biopolymers. The processing causes several by-products which are poorly utilized nowadays, i.e. they are mostly landfilled. Thus, utilization of these by-products is an important new research subject due to an increasing use of acacia tree worldwide.

One option to utilize the by-products is the energy production. Through anaerobic digestion the by-products could be used to produce biogas, which in turn could be used as a source for heat, electricity and traffic fuel.

The biogas production potential of the acacia by-products will be studied in this thesis. The aim is to see effects of different pre-treatments to enhance methane production. The challenge in using lignocellulosic material for biogas production is their structure and composition. The lignocellulosic materials are mainly composed of cellulose, hemicellulose and lignin and they are strongly linked to each other. Cellulose and hemicelluloses are degradable by anaerobic micro-organisms and can be converted to bio-methane. The structure of lignin is more complex. It protects cellulose and hemicelluloses against biological degradation. By pre-treatments the cellulose and hemicelluloses are made more accessible for the enzymatic hydrolysis, which is the first stage of the biological degradation of biomaterials. Different pre-treatments can be used to improve the hydrolysis process and, thus, the methane production.

# 2 ACACIA MEARNSII AND ITS UTILIZATION

## 2.1 *Acacia mearnsii* De Wild. (Black Wattle)

Wattle is large genus and there are 120-130 species occurring in all regions of the world except Europe and Antarctica. Most species are short-lived about 10 to 15 years. The main species planted in the world are *Acacia magium, A. saligna* and *A. mearnsii.* (Renner. 2014, 17)

*A. mearnsii* which is also called Black Wattle is endemic to Australia and its natural distribution is restricted to south-eastern Australia. It is a species of the *Fabaceae* family and currently grown in different countries around the world. *A. mearnsii* is in wide use, because it has a high potential as a result of rapid growth and short rotation (around eight years). (Schwertner Charão. 2005, 92). Nowadays South Africa and Brazil are the main cultivating countries of *A. mearnsii*. About 60% of the plantations belong to smallholders (Renner. 2014, 17). Other successful climatic conditions for *A. mearnsii* are found in other parts of Africa, Asia and South America. (Brown & Chin Ko, 1997, 15-16)

*A. mearnsii* is an evergreen, fast-growing leguminous shrub or small tree. At maturity it attains 5-15 m in height and 10-35 cm diameter at breast height depending on the form of stem. The form of the species varies with genotype, response to stand density and soil moisture availability. It occurs across hills, gullies, valleys and plains, but often the largest and best formed trees are found in places where there is an increased soil moisture availability. Species are short lived, which requires regeneration of soil. (Brown & Chin Ko, 1997, 5-6). Figure 1 shows a picture of *A. mearnsii*

Figure 1 Image of *Acacia mearnsii* (Wessa, 2010)

## 2.2 Tannin production from *Acacia mearnsii*

*A. mearnsii* is an important commercially grown tree, which is used as a raw material for many purposes. It is a fast growing and adaptable tree. The bark is used to produce tannin extract, which is used in tanning and adhesives. The hard and dense wood can be used as fuel, mine timber, tool handles and raw material for charcoal, particle board and wood pulp. It can also be used as dune stabilization and decoration and it has many favorable features for soil and water conservation. It has been shown that *A. mearnsii* has played an important role in the economic development of some countries. (Brown & Chin Ko, 1997, 3) Acacia can be mixed with pine or eucalyptus, which can bring some advantages for industries. Acacia has been especially used for pulp production, because it contains a small amount of lignin. (Renner. 2014, 18)

Tannins are polyphenolic complexes of vegetable origin, widely used in tanning leather industry, adhesives, oil, rubber and pharmaceuticals. Higher concentrations are found in wood and bark of hardwood, like *A. mearnsii*. Bark of *A. mearnsii* is most used in tannin production and about 158 000 tons of shells were extracted in the south of Brazil in 2008. Bark of *A. mearnsii* can display up to 28 % of tannins (dry weight basis). However, the content of tannins depends on several factors including genetic characteristics of the plant, climatic conditions and soil, silvicultural and management techniques. (Menezes, Marder, Ben de Costa. 2013, 2) Color ranging is

from yellow to dark brown. Lighter colored tannins are particularly important for the leather industry and therefore more valued. (Renner, R. 2014, 14)

The main advantage of producing tannins from Acacia is the age of cutting. In Brazil it varies from the age of 5 up to 10 years, while in South Africa trees are cut at the age of 11. Productivity varies between 10 to 25 $m^3$/ ha/ year. The average production of bark is around 15 t/ ha. A black wattle tree aged of 6 and 8 years old weighs about 60 kg and it contains 6 kg of peel. (Renner. 2014, 14)

Tannins are used in the manufacturing of paints, production of corrosion inhibitors, pharmaceuticals, adhesives and flocculation. They have a great importance in the manufacturing of adhesives, but they can also be used in clarification of beer and wines. The main use of tannins is skin tanning. The wattle tannin is a very versatile product and can be used in different stages of processing animal skin to leather. It is used in pre-tanning, vegetable tanning and re-tanning. The tannins are produced 350 000 t / year and the largest producers are in South Africa and Brazil. (Renner, R. 2014, 18-19)

## 2.3 Tannin production in Seta

Seta is a producer of vegetable tannins and chemical specialities. It is located in Estancia Velha, Rio Grande do Sul in the Southern Region of Brazil. It offers services to the leather industry and supplies chemical products. For water and eluent sectors, sugar and alcohol and petrochemical sectors it produces tannin-based chemicals. Seta is also working in forest industry by purchasing, promoting and improving the productivity of the black wattle/acacia trees. (Seta, 2015)

Setas subsidiary Acquaquimica has the initial focus on products for waste water and effluent treatment, like flocculants, bleachers, disinfectants and other organic specialities. Products are used in water treatment plants, municipal sewage treatment plants, dairies, textile, beverage, paper and cellulose plants etc. Products are biodegradable and they are produced from reforested trees. (Seta, 2015)

### 2.3.1 Production process of wattle extracts

After felling the trees the bole is cut into similar sizes. The shell is removed manually with knives or mechanically with a peeler. The shells are arranged in bundles and transported to the industry. The shell is evaluated based on their quality, because darker ones are used in dark tannins. The load is forwarded to weighing. Bark is removed with knives after weighing and stored manually or mechanically. Each batch is properly targeted and numbered for quality control. (Renner, 2014. 23-24) In Figure 2 bark shells are waiting for processing.

Figure 2 Bark of acacia (Kymäläinen, 2015)

At Seta company, bark is milled with a size between 5,1 and 10,0 mm. This increases the efficiency of the extraction step. In the extraction hydro solubilisation (“autoclave”) is used, where bark is operated for 8 hours at a temperature of 100 °C and pressure of 1,0 bar. The next step is removing excess water from tannin. The concentration of tannin extract (low concentration tannins, TBC) is increased with evaporation (from 10 % to 50 %). Solvent which is removed from solution is stored and re-used in extraction. Low concentration tannin (TBC) is sent to storage tanks to wait for the evaporation process. Tannin concentration is made in two evaporators. Evaporated water is sent to be reused in the extraction process. High concentration tannin extract is called TAC. (Renner, 2014. 24) In reactors TAC and other inputs are added to produce tannin. The amounts of TAC and other inputs depend of the type of product. During the reaction process parameters are monitored and analysed in a process laboratory. (Morais, 2015)

To obtain the tannin powder TAC undergoes a spray drying process, where TAC is heated in the form of spray in a hot air chamber with continuous passage at a temperature between 220 °C and 250 °C. After this, the total solids of the product is about 94 %. Pneumatic conveyors are carrying the tannin powder to be packed in 25 kg bags. Tannin can also be marketed as granulate. (Renner, 2014. 24). The whole process is presented in Figure 3.

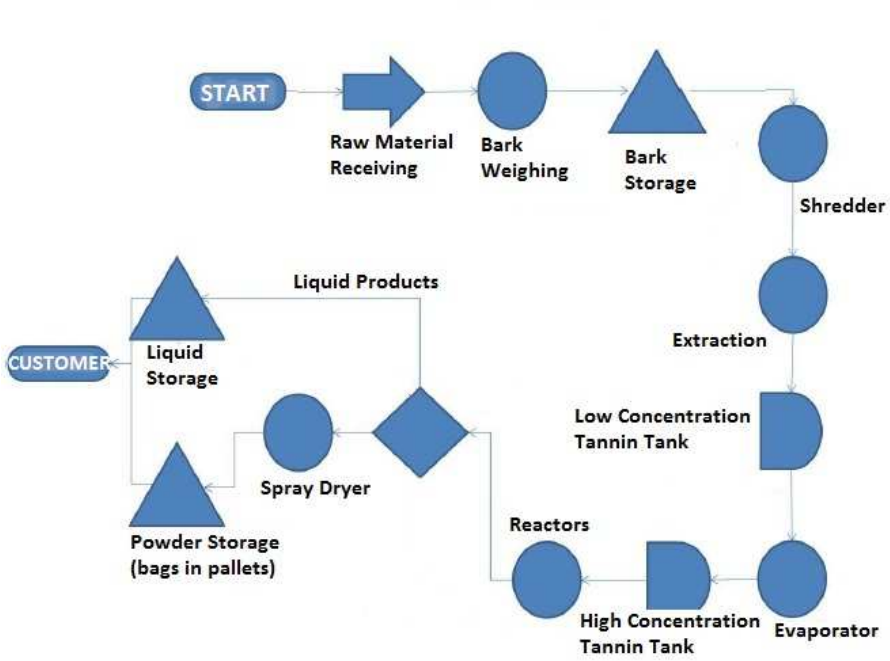



Figure 3 Process chart of tannin extracts (5.8.2015 Morais)

## 2.4 Charcoal production process

As already mentioned *A. mearnsii* is one of the most planted trees in Brazil. It is a source for tannin extracts but also a source for charcoal production. Charcoal plays an important role in Brazil as an energy source. Charcoal is obtained by a process known as pyrolysis. If wood is used here as a raw material the charcoal is a renewable energy source. The demand for charcoal is expected to increase in the coming years. (Froehlich & Moura. 2014, 2)

Charcoal is produced as an energy carrier e.g. for cooking and heating. Biochar is produced for applications to soil as a part of agronomic or environmental management. Generally, charcoal and biochar are carbonaceous material and they are produced by the thermal decomposition of organic material at a high temperature (350-1200°C). Generally, charcoal refers to wood biochar, but biochar can be produced from other biomass and even from processed biomass (e.g. paper mill waste). (Lehmann. 2015, 15).

Pyrolysis is the thermochemical process, which converts biomass into usable fuels. Biomass is applied in high heat in the absence of air and this process results in charcoal, condensable organic liquids (pyrolytic fuel oil), non-condensable gases, acetic acid, acetone and methanol. Pyrolysis has been used since the dawn of civilization. Pyrolysis process is shown in Figure 4. (Osburn & Osburn, 1993)

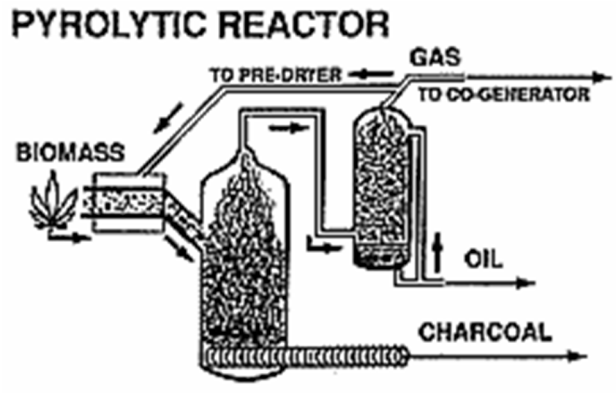



Figure 4 Pyrolytic reactor (Osburn & Osburn, 1993)

The charcoal from the pyrolysis of the *A. mearnsii* wood is processed into bricks to be used in heating and cooking ovens, but gas is released into atmosphere. The charcoal industry is responsible for discharging materials, which can pollute the surrounding environment. The wood charcoal production is still very rudimentary and it generates a high amount of disposable residues. It is known that under controlled conditions, e.g. the gas could be utilized in many ways. By condensing the gas, it is possible to get pyroligneous liquid that can be utilized as a raw material for pharmaceutical, cosmetic and oil industries and other applications. (Furtado, dos Santos Stolz, Pinto, Moura, Dal Pont Morisso, Pitarelo, Ramos, von Mühlen, Riegel-Vidotti. 2015, 1-2)

# 3 ANAEROBIC DIGESTION OF LIGNOCELLULOSIC MATERIAL

## 3.1 Anaerobic digestion

Anaerobic digestion is an effective waste treatment and sustainable energy production method. Biogas production from municipal, agricultural and industrial wastes can contribute sustainable energy production and it does not make competition for land use for food production. (Wall, Harwood & Demain. 2008, 195). Biogas is mostly a mixture of methane ($CH_4$) and carbon dioxide ($CO_2$) and it can be produced from organic matter via anaerobic digestion. Typically, biogas contains 50-75 % methane, 25-50 % carbon dioxide, 1-5 % water vapour and 0-5 % nitrogen. It can contain a small amount of hydrogen sulphide (0-5,000 ppm) and ammonia (0-500 ppm). (Frigon & Guiot. 2010, 447)

After anaerobic digestion (AD) solid digestate can be used as a peat-type organic amendment for soil. Concentrated nutrient-rich effluent can be used as a fertilizer on agricultural fields. (Frigon & Guiot. 2010, 447)

Anaerobic digestion has four different steps: hydrolysis, acidogenesis, acetogenesis and methanogenesis. Each step has its own specific group of microorganisms. Hydrolysis converts carbohydrates into soluble sugar monomers (glucose, arabinose, mannose and xylose). In acidogenesis soluble sugars monomers transform into volatile fatty acids (VFA). During

acetogenesis, VFAs are transformed into acetate, $CO_2$ and $H_2$. In methanogenesis, hydrogenophilic methanogens transform the mixture of $CO_2/H_2$ into methane and acetoclastic methanogens transform acetate into methane. Anaerobic digestion is sensitive to environmental factors like temperature, pH and toxic compounds. AD process is divided into psychrophilic (10-20°C), mesophilic (20-40°C) and thermophilic (50-60°C) digestion processes. The first stages in AD process can occur at a wide range of pH, but methanogenic microorganisms are sensitive to pH changes and neutral pH (6,5-7,5) is efficient for them. (Monlau, Barakat, Trably, Dumas, Steyer & Carrere. 2013, 289-292)

Biogas can be made from animal manure, most biomass and organic waste materials. Feedstock for this process can be composed of carbohydrates, proteins, fats, lignocellulosic mass and mixtures of these. Also, the moisture of feedstock can vary and wastes can be solid, slurry or liquid. (Wall, Harwood & Demain. 2008, 195) In general, in the fermentation process there should be C/N ratio in fermentable mass between 25 and 35. The growth of industrialization and urbanization has increased the quantity of sludge from waste water treatment plants (WWTPs). The sewage sludge has a C/N ratio between 6:1 and 9:1 and mixing sewage sludge with the municipal solid waste or food waste can increase the C/N ratio to favourable for anaerobic digestion. (Ackmez. 2012, 4)

## 3.2 Lignocellulosic biomass

Lignocellulosic biomass represents the vast bulk of plant material and it includes agricultural waste (straw, corn stover etc.), forestry wastes, a fraction of municipal and industrial paper wastes and fast growing energy crops like miscanthus or switchgrass. Typically, biomass contains on the dry weight basis 40-60 % cellulose, 20-40 % hemicelluloses and 10-25 % lignin. (Wertz, Bedue. 2013, 16)

The cellulose and hemicellulose fractions are carbohydrates and lignin is a complex phenolic compound. Lignocellulosic biomass also includes water and a small amount of proteins and other compounds. Cellulose has both amorphous and crystalline structures and the cellulose strains are bonded together with cellulose fibrils. Hemicellulose has micro- and macrofiborous between cellulose. Lignin is like matrix, where cellulose and hemicellulose are embedded. (Gubta, Vijai, Tuohy- 2013, 5) Holocelluloses (cellulose and hemicelluloses) have been shown to be biodegradable in their pure form, but content of lignin creates challenges in anaerobic digestion, because it is hydrophobic polymer and it is fairly resistant to anaerobic digestion. (Monlau, Barakat, Trably, Dumas, Steyer & Carrere. 2013, 261-262) The structure of lignocellulosic material is presented in Figure 5.

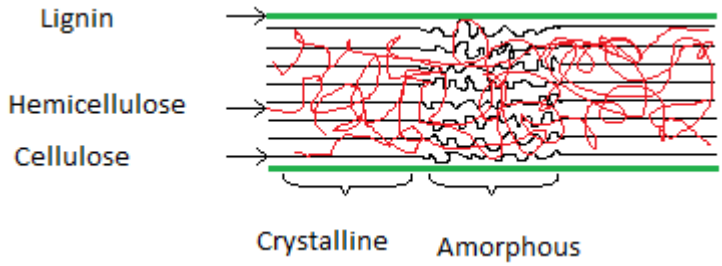



Figure 5 Lignocellulosic material. (Mosier ect. 2004, 674)

### 3.2.1 Cellulose

Cellulose is the most abundant polysaccharide on earth and it represents over 50 % of the wood mass. The chemical formula is $(C_6H_{10}O_5)_n$. It contains β-1,4 glucosidic bonds between glucose molecules and it forms long straight chains. Hydroxides are distributed on both sides of the monomers. Numerous strong intermolecular hydrogen bonds between hydroxyl groups form molecules in parallel chains. (Gubta, Vijai, Tuohy- 2013, 7) Cellulose is insoluble in water and in most organic solvents. With an acid and thermal treatment, it can be broken down into glucose. (Monlau, Barakat, Trably, Dumas, Steyer & Carrere. 2013, 265)The structural unit of cellulose is presented in Figure 6.

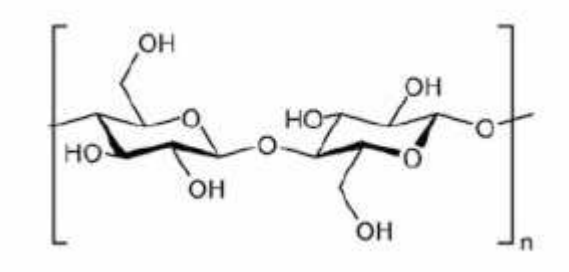



Figure 6 Structural unit of cellulose (Gupta, Tuohy. 2013, 6)

### 3.2.2 Hemicelluloses

Hemicelluloses consists of different monosaccharide units. Polymer chains have short branches and they are amorphous. Thus, they are partially soluble or swell in water. Polymer chains can consist of single sugar repeat unit (homopolymer) or they can be a mixture of different sugars (heteropolymer). The most important sugar of hemicelluloses is xylose. (Gubta, Vijai, Tuohy- 2013, 7) Hemicelluloses also includes arabinose, glucose, mannose, galactose, galacturonic acid and glucuronic acid. The most important role of hemicelluloses is to strengthen the cell wall by interaction with cellulose and lignin. (Wertz & Bedue. 2013, 243) From all lignocellulosic components, hemicelluloses are the most thermal-chemically sensitive. (Monlau, Barakat, Trably, Dumas, Steyer & Carrere. 2013, 265). The backbone of hemicelluloses and formulas of the sugars in hemicelluloses are presented in Figures 7 and 8.

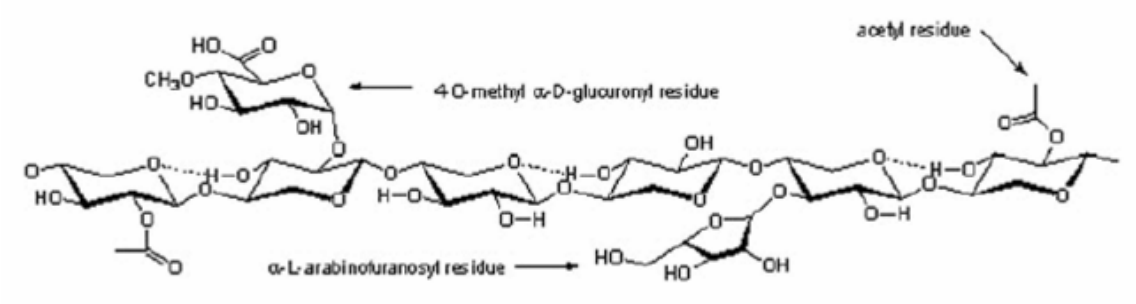



Figure 7 Schematic representation of the hemicellulose backbone (Harmsen et al. 2010)

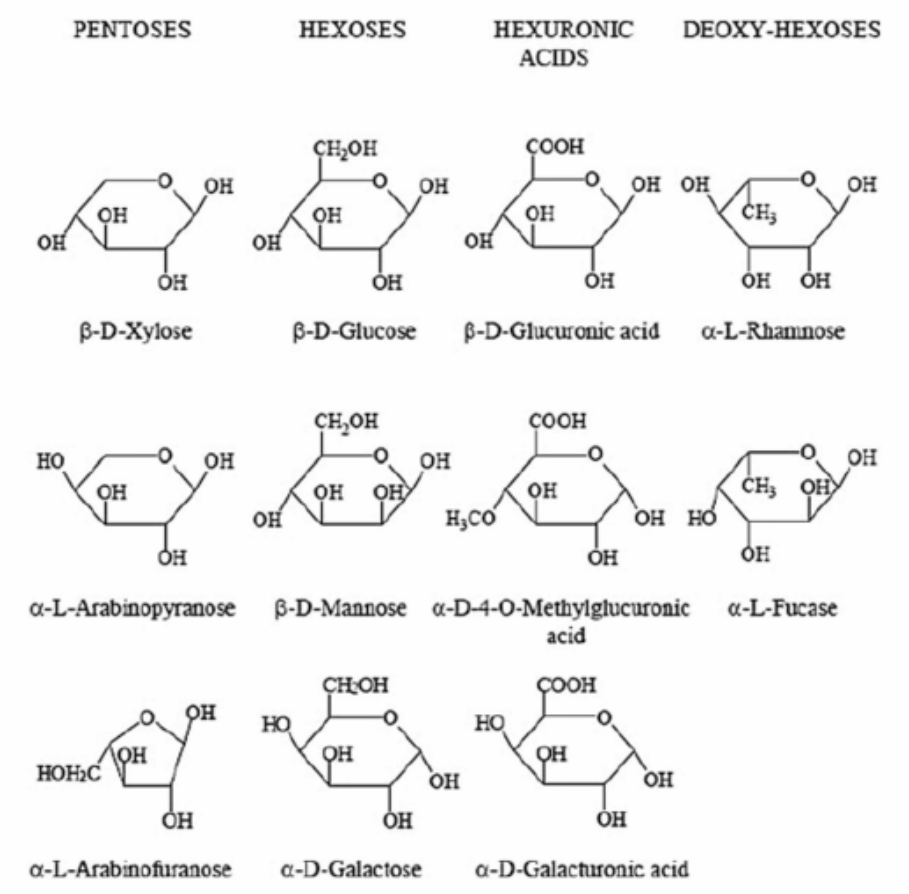



Figure 8 Formulas of the sugar component of hemicelluloses (Mustafa et al 2009)

### 3.2.3 Lignin

Lignin is a complex phenolic polymer, which gives strength and hydrophobicity to plant secondary cell walls. Lignin is highly resistant to enzymatic and mechanical degradation. Lignin polymers make the cell wall impervious, allowing the transport of water and nutrients through and protecting the plant against microbial invasion. Lignin composition varies among cell types and it can be different in individual cell wall layers. The composition is also influenced by environmental conditions. (Wertz & Bedue. 2013, 262)

The composition of lignin is important to understand to enhance the digestibility of biomass. Depolymerization and repolymerization of lignin molecules is a very important parameter for the biodegradability of lignocellulosic biomass. Lignin consists of three different phenylpropane alcohols: p-coumaryl (H), coniferyl (G) and sinapyl (S) and the quantity of these alcohols varies between different biomass like hardwood, softwood or herbaceous. (Monlau, Barakat, Trably, Dumas, Steyer & Carrere. 2013, 266-267) The structure of lignin is presented in Figure 9.

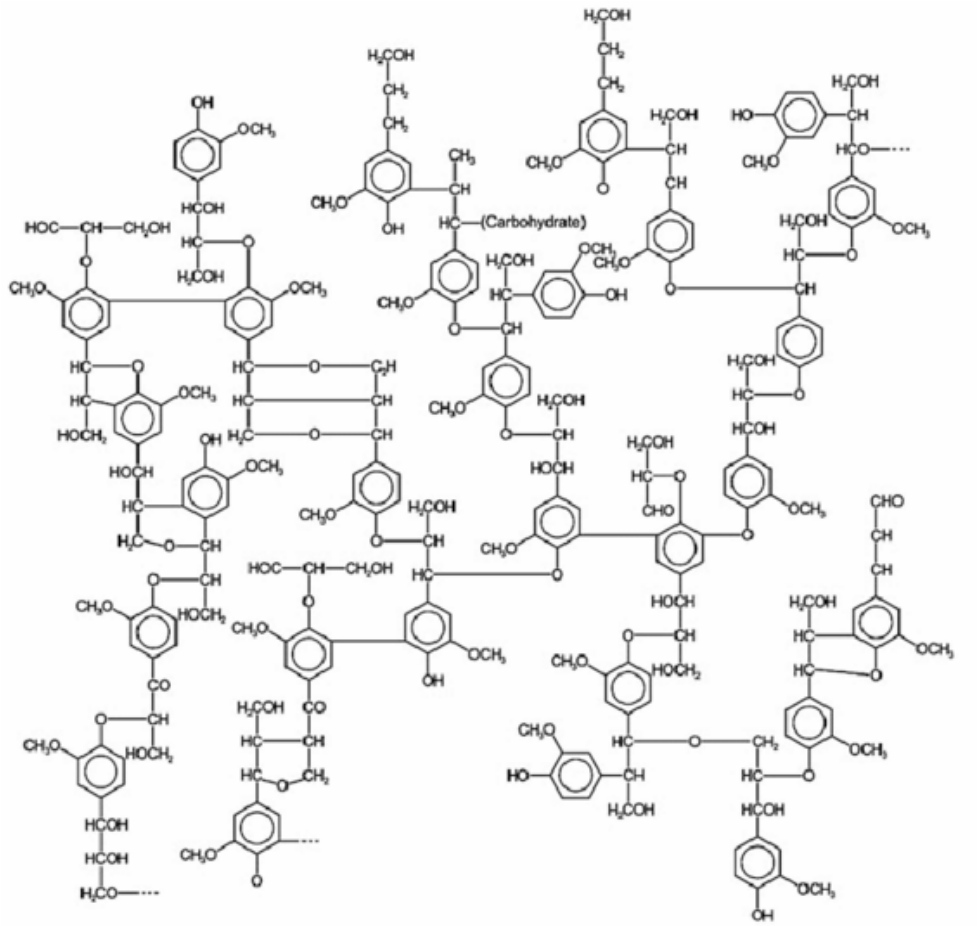



Figure 9 Structure of lignin (Glazer et al. 1995)

### 3.2.4 Lignocellulosic biomass in anaerobic digestion

The theoretical or maximum methane yield can be calculated from the composition of substrate $C_aH_bO_cN_dS_e$ known as Buswell's equation (as follows figure 10):

$$Y_{CH_4}^{Theoretical}(L/g_{substrate}) = \frac{22.4\left(4a+b-2c-3d-2e\right)}{8\left(12a+b+16c+14d+16e\right)}$$

Figure 10 Theoretical methane yield (Monlau, Barakat, Trably, Dumas, Steyer & Carrere. 2013, 292)

Typically, the actual and experimental methane yields are far lower than the theoretical ones. This is because biomass consists of nonbiodegradable compounds (lignin, peptidoglycan) and polymers that are difficult to dissolve. Many reviews have been published on methane production from different biomass: In Table 1 the results of one review are given.

Table 1 Review of methane production from different biomass (Monlau, Barakat, Trably, Dumas, Steyer & Carrere. 2013)

| Substrate | Methane yield (L CH/kg $_{VS\ added}$) | Energy yield (MJ/kg $_{VS\ added}$) |
|---|---|---|
| Newsprint | 97 | 3,86 |
| Corn stover | 114 | 4,54 |
| Wheat grass | 160 | 6,37 |
| Rice straw | 194 | 7,72 |
| Willow | 200 | 7,96 |
| Miscanthus | 200 | 7,96 |
| Paper tube residues | 222 | 8,83 |
| Grass hay | 230 | 9,15 |
| Wheat straw | 276 | 10,98 |
| Sugar beet tops | 310 | 12,33 |
| Potatoes | 328 | 13,05 |
| Office paper | 364 | 14,48 |
| Maize sillage | 370 | 14,72 |
| Citrus peels | 455 | 18,1 |

The lignin and fiber content influences the methane production by limiting the access to holocelluloses, because they are less biodegradable when they are combined with lignin. In anaerobic digestion the conversion from lignocellulosic biomass to biogas is strongly linked to bioaccessibility of cellulose. Thus, lignin concentration is a key parameter in anaerobic biodegradation. (Monlau, Barakat, Trably, Dumas, Steyer & Carrere. 2013, 289-293)

## 3.3 Pre-treatment for enhancing methane production

Lignin linkages between cellulose and hemicelluloses prevent their degradation. With pre-treatments it is possible to break down the linkages between polysaccharides and lignin thus making cellulose and hemicelluloses more accessible to hydrolytic enzymes. Pre-treatments can accelerate the hydrolysis process and then improve methane production. (Sambusiti, Monlau, Ficara, Carrère, Malpei. 2013, 63)

The pre-treatment can be a mechanical, chemical, thermal and biological process or a combination of them. Mechanical pre-treatment is, for instance, size reduction which makes substrates more biodegradable by rupturing cell walls. Methods are usually mechanical jet, high pressure homogenizer, mechanical ball mill etc. Separation by size is also one form of mechanical pre-treatment. For thermal pre-treatment the optimum temperature and duration

depend largely on the nature of the substrate. The temperature above 200 °C could promote an inhibitory effect on the digestion process. Also, ultrasound is one of the mechanical pre-treatment forms. (Ackmez. 2012, 7)

Chemical pre-treatments are classified into acidic, alkaline, organosolv, inorganic salts, oxidative, ammonia and ionic liquids pre-treatments. Alkaline and acidic ones are the most studied and sulphuric acid is the most applied acid. An acid pre-treatment is used for removing hemicelluloses by breaking ether bonds in lignin/phenolic-carbohydrates complex. In alkaline pre-treatments the bases, such as sodium, potassium, calcium and ammonium hydroxides are used. They are effective to alter the structure of lignin and increase the enzymatic accessibility to cellulose and hemicelluloses. Calcium hydroxide or sodium hydroxide pre-treatments were shown to be effective at a lower temperature (15-55 °C) but they need more time than acid pre-treatments. Different thermo-alkaline pre-treatments with sodium hydroxide at different temperatures has been studied with agro-wastes. Commonly used agricultural substrates in thermoalkaline pre-treatments have been cornstalks, corn stover, rice straw, sweet sorghum stalks, grasses, sunflower stalks, barley waste and soybean straw. (Sambusiti, Monlau, Ficara, Carrère, Malpei. 2013, 63)

In the biological pre-treatment the aim is to prepare the substrates for the enzymatic degradation. The method and conditions depend greatly on the type of substrate. Several fungi and bacteria have been used for this purpose. The benefits of the biological pre-treatment are low energy requirement, no chemical requirement and mild environmental conditions. The treatment efficiency is still very low. (Ackmez. 2012, 18)

# 4 MATERIAL AND METHODS

Residues of acacia processing by-products have never been tested for biogas production and it will be done in this thesis. Their composition has been studied and it can be expected that an alkali pretreatment will improve the anaerobic digestion process.

## 4.1 Extraction residue, sludge and bark

The materials tested were supplied by a Brazilian tannin producer named Seta, which produces tannin extracts for biopolymers from *Acacia mearnsii*. Three different fractions, i.e. sludge, extraction residue and original bark were tested. The sludge represents precipitated liquid fraction from extraction. The solid part of the extraction is called extraction residue and the third sample is untreated bark. Bark was a reference for sludge, residue and pretreated samples. The samples by Seta are shown in Figure 11.


Figure 11 Extraction residue (1), bark (2) and sludge (3).

The current utilization of extraction residue is used as a boiler fuel to make steam to the tannin extract process. The sludge is sent to certified areas, which utilize sludge for agricultural soils. (Morais, 2015)

## 4.2 Pyroligneous liquor

The fourth studied sample was pyroligneous liquor which is composed in the charcoal process. Pyroligneous liquor samples were delivered from Brazil Research group in Feevale University who have concisely studied the composition of pyroligneous liquor from *Acacia mearnsii*. The composition study revealed that the extract consists of complex chemicals and they are derived from the thermal degradation of *Acacia mearnsii* lignocellulosic constituents. The extract contains various phenolic compounds which are derived from lignin reactions. The extract also contains oxygenated compounds which come from secondary radical reactions. Also, compounds with lower molecular mass were detected. The samples were also very acidic. (Furtado, dos Santos Stolz, Pinto, Moura, Dal Pont Morisso, Pitarelo, Ramos, von Mühlen, Riegel-Vidotti. 2015, 4-7)

Liquid pyroligneous liquor samples were not pre-treated prior to methane production tests. The chemical oxygen demand of pyroligneous liquor was determined before the test to calculate the amount of other substrates for the biochemical potential methane test (BMP).

## 4.3 Analytical methods

Total and volatile solids (TS, VS), hemicellulose, cellulose lignin and phenols were analysed from the samples. COD (chemical oxygen demand) was determined from pyroligneous liquor. In Figures 12, 13 and 14 there are descriptions of methods and analyses used for each sample.

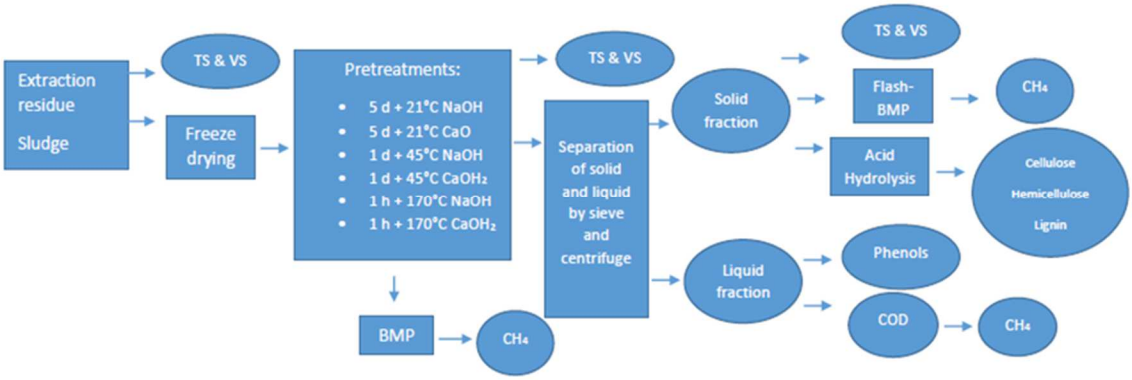



Figure 12 Description of methods and analyses for extraction residue and sludge samples (pre-treatment process)

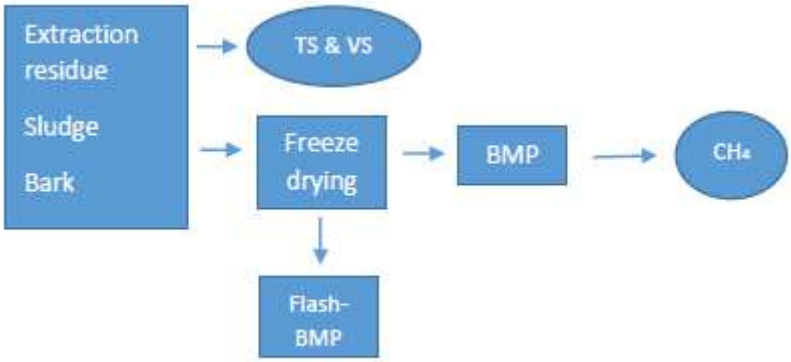



Figure 13 Description of methods and analyses for original acacia samples

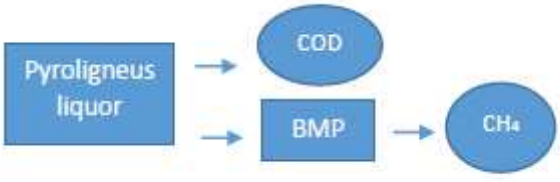



Figure 14 Description of methods and analyses for pyroligneous liquor

### 4.3.1 Total solids and volatile solids from original samples

Total solids (TS) and volatile solids (VS) of the original samples were determined before freezing the samples. The samples were weighed to tared

and weighed crucibles. The samples were dried for 24-48 hours at 105°C. After drying, crucibles with samples were weighed and samples were incinerated at 550 °C. TS and VS results of original samples are presented in Table 2.

Table 2 TS and VS of original samples

| Sample | TS (%) | σTS (%) | VS (%) | σVS (%) | VS/TS |
|---|---|---|---|---|---|
| Extraction recidue | 44,3 | 0,6 | 41,6 | 0,8 | 94 |
| Sludge | 17,3 | 0,0 | 15,9 | 0,0 | 92,1 |
| Original bark | 53,9 | 2,6 | 51,8 | 2,8 | 96,1 |

After TS and VS determination the samples were frozen in a freeze dryer. The principle of a freeze dryer is to remove water by sublimation from the frozen state. The samples must be first frozen and then subjected to high vacuum. Water is evaporated without melting. The released water vapour is condensed on the surface of a condenser at a low temperature. Freeze drying does not cause thermal damage compared to other drying options. The freeze dryer which was used was HetoPowerDry PL 3000: ThermoElectron Corporation. (Berk, Z. 2013, 567) Figure 11 shows the original samples from Seta. On the top there is extraction residue, in the middle there is bark and at the bottom there is sludge.

TS and VS was measured after freeze drying and the results are given in Table 3.

Table 3 TS and VS of freeze dried samples

| Sample | TS (%) | σTS (%) | VS (%) | σVS (%) | VS/TS |
|---|---|---|---|---|---|
| Extraction recidue | 97,2 | 0,3 | 91,0 | 0,7 | 93,6 |
| Sludge | 96,2 | 0,3 | 88,7 | 0,3 | 92,2 |
| Original bark | 97,0 | 0,1 | 93,1 | 0,2 | 96 |

### 4.3.2 Determination of hemicellulose, cellulose and lignin

The amount of cellulose, hemicellulose and lignin were determined from the original and pre-treated samples, to see the effect of alkaline pre-treatment. Components were analysed according to the National Renewable Energy Laboratory analytical procedure (NREL, LAP) methods (determination of Structural Carbohydrates and Lignin in Biomass) (Sluiter ect. 2011). In this method acid hydrolysis is used to hydrolyse lignocellulosic material into monosaccharides. Acid hydrolysis was made from solid fractions of samples.

Before acid hydrolysis, bark was extracted with ethanol. The extraction was based on an analytical procedure: “Determination of Extractives in Biomass”. The extraction was made to remove ethanol soluble material. (Sluiter ect. 2005) The extraction was made for bark only, because other samples had already been extracted in the tannin process.

Sulphuric acid ($H_2SO_4$) was used to hydrolyse carbohydrates from lignocellulose and further carbohydrates into monosaccharides (glucose, xylose, mannose, arabinose, galactose, fructose). The determination of monosaccharides was done by a high-performance liquid chromatography (HPLC). This HPLC consists of an automatic sampler (Water 717), a pre-column to filter residues (Micro guard cation H refill cartridge, Bio-rad) and a Aminex HPX-87H column (300 mm on 7, 8 mm, Bio-rad). The determination was made by using sugar column for sugar standards (glucose, xylose, arabinose, glycerol) and acetone. The carrier liquid was sulfuric acid at 0.005 M at 0.4 mL/min. The detector was Refractive Index (RI). HPLC samples were prepared by passing the decanted liquid through a 0,2 μm nylon filter into a vial. The samples were stored in a freezer prior to determination.

Lignin was also determined in the acid hydrolysis procedure. After acid hydrolysis, the samples were filtered and acid-insoluble lignin was gravimetrically determined from solid fraction by drying it in the oven at a temperature of 105 °C. After drying solids were weighed and then they were incinerated in an oven at 550 °C. After burning samples were weighed again. Cellulose (1), hemicelluloses (2) and acid soluble lignin (AIL) (3) were calculated as shown in equations

$$\% \text{ cellulose} = \frac{[\text{glucose}]}{\text{m1ODW}} \times 0{,}9 \qquad (1)$$

where:
0,9 = anhydro correction
m1ODW = Oven dry weight of sample

$$\% \text{ hemicellulose} = \frac{[\text{xylose}] + [\text{arabinose}]}{\text{m1ODW}} \times 0{,}88 \qquad (2)$$

where:
0,88 = anhydro correction
m1ODW = Oven dry weight of sample

Acid soluble lignin:

$$\% \text{ AIL} = \frac{(m5 - m4) - (m6 - m4)}{m0} \times 100 \qquad (3)$$

where:
$m5 = \text{Weight}_{\text{crucible + insoluble residue}}$
$m4 = \text{Weight}_{\text{crucible}}$
$m6 = \text{Weight}_{\text{crucible + ash}}$
m0 = oven dry weight (ODW) of sample

Compositions of the original samples are given in Table 4.

Table 4 Amount of cellulose, hemicellulose and lignin in original samples

| Sample | Cellulose %/TS | σ Cellulose (%) | Hemicellulose %/TS | σ Hemicellulose (%) | Lignin %/TS | σ Lignin (%) |
|---|---|---|---|---|---|---|
| Exraction residue | 29,7 | 0,4 | 13,4 | 1,0 | 38,6 | - |
| Sludge | 8,4 | 0,5 | 10,4 | 0,3 | 41,0 | 2,2 |
| Bark | 28,1 | 2,2 | 14,7 | 2,3 | 39,5 | 0,7 |

### 4.3.3 Phenols

As earlier presented, lignin is a natural source of phenolic compounds. The determination of phenols was made to see the effectiveness of alkaline pre-treatments. Phenols were in the liquid fraction of the pre-treated samples and were determined spectrophotometrically. The result indicates the presence of an aromatic ring with hydroxyl groups, which can be free or engaged with a carbohydrate. This technique is based on the action of Folin Ciocalteu reagent, mixture of phosphotungstic acid and phosphomolybdic acid. The reaction produces blue colour when phenols are oxidized. The generated colour can be measured spectrophotometrically at 735 nm and number can be convert as the amount of phenols in the analysed sample.

The phenols were analysed from the liquid fraction of the pre-treated samples. Each of them was analysed in triplicate, but some of the samples were over the range and some of the samples had to be measured several times. The phenol concentration of the sample was determined based on the calibration curve. The calibration curves are presented in Appendix 3.

### 4.3.4 Determination of COD from pyroligneous liquor

Chemical oxygen demand (COD) measures the amount of organic compounds in a liquid solution. It is expressed gram of COD per liter (gCOD/L) and it indicates the mass of oxygen consumed per liter of solution. COD was determined from liquid part of the pre-treated samples and from pyroligneous liquor. COD was determined by using Hach COD Test'N Tubes 0-1500 mg/l $O_2$. COD of pyroligneous liquor was very high as expected. The amount was 99,5 g/L and this was observed, when BMP bottles were filled.

## 4.4 Test methods

### 4.4.1 Pre-treatments

Alkaline pre-treatments are effective in altering the structure of lignin. Alkaline pre-treatments have been studied for many years in order to increase methane production. In this study three different alkaline chemicals were used: sodium hydroxide (NaOH), calcium oxide (CaO) and calcium hydroxide ($Ca(OH)_2$). Sodium hydroxide treatment is also known as soda-treatment and calcium oxide and calcium hydroxide is known as lime-treatment.

Calcium oxide was used in low temperature and calcium hydroxide was used in higher temperatures.

Pre-treatment conditions used in this thesis are presented in Tables 5 and 6. All pre-treatments were done in 500 ml flasks with a chemical agent concentration of 10 % (weight-% of dry solids) or with 13,2 % (weight-% of dry solids) with a solid concentration of 50 gTS/L (=5 %) . Bottles were closed with rubber sept and made fourfold, i.e. three for the BMP test and one for the chemical analysis.

Table 5 Pre-treatment conditions of extraction residue sample

| Alkali | Weight % of TS | Temperature °C | Duration |
|---|---|---|---|
| NaOH | 10,0 | 21; 45; 170 | 5 d; 24 h; 1 h |
| CaO | 13,2 | 21 | 5 d |
| $CaOH_2$ | 13,2 | 45; 170 | 24 h; 1 h |

Table 6 Pre-treatment conditions of sludge sample

| Alkali | Weight % of TS | Temperature °C | Duration |
|---|---|---|---|
| NaOH | 10,0 | 21; 45; 135 | 5 d; 24 h; 1 h |
| CaO | 13,2 | 21 | 5 d |
| $CaOH_2$ | 13,2 | 45; 135 | 24 h; 1 h |

Pre-treatments were made at different temperatures and duration was 1 hour, 24 hours and 5 days depending on the pre-treatment. The treatments at 21 °C and 45 °C were performed on a shaker with an agitation of 150-160 rpm. Treatment at 135 °C was performed in an autoclave. Pre-treatments were done in BMP bottles except the pre-treatment at 170 °C which was performed by using a stainless steel autoclave, with a capacity of 1 L. The rotation was around 160 rpm and samples were heated for 1 hour. After heating the samples and water were collected from the autoclave to the vessel and cooled. The stainless steel autoclave used in this thesis is presented in Figure 15

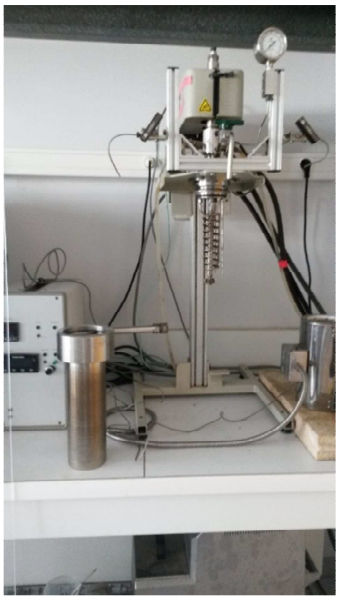


Figure 15 The stainless steel autoclave used in the thesis

After pre-treatments BMP tests were started with three bottles and one bottle was used for the chemical analyses. For analytical purposes solid and liquid parts of the pre-treated sample were separated by using a sieve and centrifuge. After centrifugation the supernatant was filtered with a glass microfiber filter (Grade GF/D: 2,7 µm). The solid part was dried at 40 °C and stored at a room temperature. The filtered liquid part was stored in a fridge.

### 4.4.2 Biochemical methane potential (BMP) test

The inoculum, i.e. active anaerobic sludge, for BMP tests was prepared in an anaerobic reactor of 5 liters (Figure 16). The sludge used as a reactor feed was from a waste water treatment plant of sugar factory and was regularly fed with ethanol. The activity of inoculum was confirmed with an inoculum test, which was made in three 500 ml bottles. The bottles were filled with a macro nutrient solution, an oligoelement solution, buffer solution and the inoculum sludge. A small amount of ethanol was added to two bottles to start methane production. One bottle was without ethanol and it was the "blank" one. The test bottles were flushed with nitrogen ($N_2$)–gas to ensure the anaerobic conditions. The bottles were stored 2 days at 35 °C in a mixing table and gas production was tested after a couple of days to make sure that inoculum is working.

Figure 16 Anaerobic reactor for the preparation of inoculum for BMP test

TS and VS content of the samples and inoculum sludge were determined before making the BMP test bottles. TS content of the inoculum was 98 gTS/L and VS was 45 gVS/L. The VS-result was used to calculate the amounts of inoculum, sample and nutrients for BMP test bottles. The bottles were filled as inoculum test bottles. Both tests contained 27 bottles (2 x 18 bottles from pre-treatments, 12 original samples and 2 x 3 blank samples). The weighted amounts for each substrates are given in Appendix 1/1 and 1/2.

BMP test was performed under mesophilic conditions (35 °C +/- 0,5 °C). The tests were performed in 500 ml glass bottles with rubber septa in batch mode (Figure 17). The working volume of the bottle was 400 ml. The bottles were kept in a mixing table (Figure 18). Stirring rate of mixing table was 80 rpm. BMP tests were performed in triplicate and test duration was 4 months.

Figure 17 500 ml glass bottle with rubber septa, manometer and syringe

Accumulated gas production was measured with a manometer and the compositions of gas were analysed with a gas chromatography. First, the measurements were done every second day, and afterwards less frequently depending on the gas production. The samples for gas chromatography were taken with a hypodermic syringe.

Figure 18 Mixing table for BMP bottles

### 4.4.3 Flash BMP

The Biochemical Methane Potential (BMP) test is widely used as a parameter for waste characterisation. It measures the quantity of methane, what waste can potentially be produced in anaerobic conditions. However, BMP test lasts for a long time, about 30 days and up to several months. Thus, it is not a useable method for an industrial use for anaerobic digestion optimization, where a user would like to find out a potential mix from different kind of waste categories. (Lesteur ect. 2011, 2280-2281)

There are several techniques to predict the BMP value faster than the biochemical way. In this thesis near infrared spectroscopy (NIRS) was used to compare the results from BMP-test. (Lesteur, M ect. 2011, 2280-2281)

NIRS is an analytical method which is based on interactions between photons (1000-2500 nm) and the matter. There is a calibration model, which is created and used to find out a relationship between the spectra and value of interest. NIRS can predict either quantitative or qualitative data and it has been used to predict organic matter components in several kind of matrix (fruits, vegetables, forages, soils). NIRS could be used in predicting the digestibility of different organic matter and INRA has developed a way to predict the BMP value of wastes. (Lesteur ect. 2011, 2280-2281)

All the analysed samples were freeze dried and ground to improve the homogeneity of the final samples. The samples for the flash BMP test were the pre-treated samples, original bark, sludge and extraction residue. Two different grinders were used: a knife grinder IkaWerke MF 10 basic and a centrifugal grinder Fritsch Pulverisette 14. However, acacia is hard material

and its fibres didn't break with those devices. Thus, a ball mill was used to grind the material into powder.

# 5 IMPACT OF PRETREATMENT ON SAMPLE CHARACTERISTICS

Changes in composition after the pre-treatment were one evaluation criterion for a sufficient pre-treatment efficiency. The aim was to liquefy solid to liquid without degrading glucose. Therefore, solubilisation of solid fraction and reduction of cellulose, hemicelluloses and lignin were analysed. Phenols and COD were determined from liquid fraction.

## 5.1 TS & VS

After pre-treatments solid and liquid fractions were separated. The solid fraction was dried at a temperature of 60 °C and TS and VS were determined after drying. The results of TS and VS of the pre-treated samples are shown in Tables 7 and 8.

Table 7 TS and VS results after pre-treatment in extraction residues

| Pretreatment | TS (%) | VS (%) | VS/TS |
|---|---|---|---|
| NaOH21 | 94,2 | 79,3 | 84,2 |
| CaO | 91,8 | 74,2 | 80,8 |
| NaOH55 | 94,2 | 81,2 | 86,2 |
| CaOH55 | 92,9 | 77,8 | 83,7 |
| NaOH170 | 95,3 | 83,6 | 87,7 |
| CaOH170 | 92,5 | 76,6 | 82,8 |

Table 8 TS and VS results after pre-treatment in sludge

| Pretreatment | TS (%) | VS (%) | VS/TS |
|---|---|---|---|
| NaOH21 | 91 | 77,9 | 85,6 |
| NaOH55 | 93,5 | 80,5 | 86,1 |
| NaOH170 | 94,6 | 74,5 | 78,8 |
| CaO | 91,7 | 74,3 | 81,0 |
| CaOH55 | 90,8 | 74,5 | 82,0 |
| CaOH135 | 93,7 | 75,3 | 80,4 |

## 5.2 TS solubilisation

The amount of samples before and after pre-treatment was weighed. With a difference, it was possible to calculate solubilisation of solids under the pre-treatment. Because of a small quantity of samples and problems with separation, mass results from the pre-treatment NaOH21 of sludge sample are not reliable and are therefore not presented. Pre-treatments at a temperature

of 170 degrees Celsius were also challenging and the results from these pre-treatments are directional. The results of TS solubilisation are shown in Figure 19 and 20. Changes in dry weight are presented in Appendix 2.

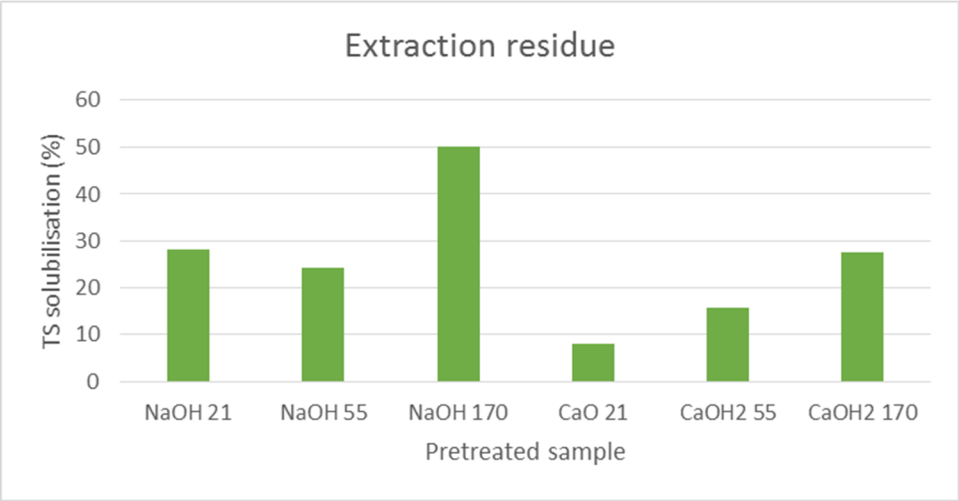



Figure 19 TS solubilisation of extraction residue sample in pre-treatment

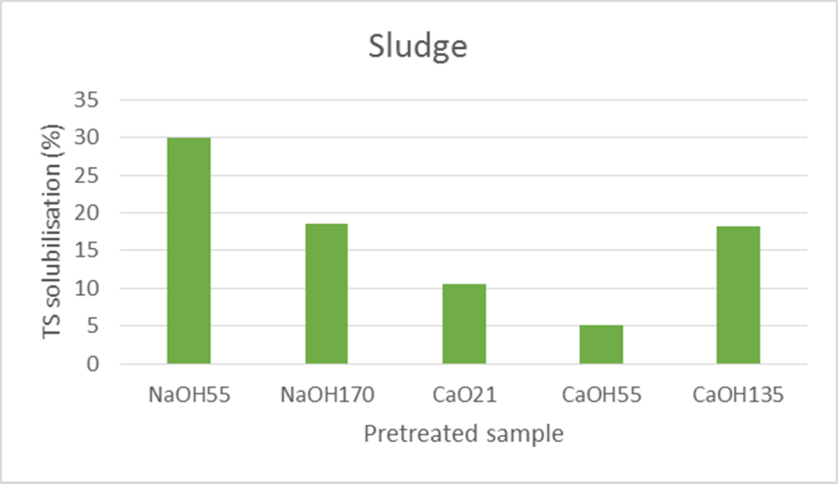



Figure 20 TS solubilisation of sludge sample in pre-treatment

## 5.3 Characterisation of solids after pre-treatment

Cellulose, hemicelluloses and lignin were determined from a solid fraction of the pre-treated sample. The solid fraction was separated from liquid fraction and dried at a temperature of 65 °C. The amount of cellulose, hemicellulose and lignin were determined by an acid hydrolysis method. The results are presented in Tables 9 and 10.

Table 9 Amount of cellulose, hemicellulose and lignin from extraction residue samples

| Extraction residue samples | Cellulose %/TS | σ Cellulose (%) | Hemicellulose %/TS | σ Hemicellulose (%) | Lignin %/TS | σ Lignin (%) |
|---|---|---|---|---|---|---|
| Original | 29,7 | 0,4 | 13,4 | 1,0 | 38,6 | - |
| NaOH21 | 35,2 | 1,3 | 13,2 | 0,3 | 22,8 | 5,0 |
| NaOH55 | 37,0 | 1,0 | 12,5 | 2,1 | 20,3 | 3,2 |
| NaOH170 | 44,9 | 3,2 | 11,2 | 0,0 | 22,2 | 1,7 |
| CaO21 | 31,0 | 3,6 | 11,7 | 0,8 | 24,6 | 0,9 |
| CaOH55 | 27,8 | - | 10,9 | - | 27,0 | 6,8 |
| CaOH170 | 35,7 | - | 10,6 | - | 30,2 | - |

Table 10 Amount of cellulose, hemicellulose and lignin from sludge samples

| Sludge samples | Cellulose %/TS | σ Cellulose (%) | Hemicellulose %/TS | σ Hemicellulose (%) | Lignin %/TS | σ Lignin (%) |
|---|---|---|---|---|---|---|
| Original | 8,4 | 0,5 | 10,4 | 0,3 | 41,0 | 2,2 |
| NaOH21 | 11,2 | 1,2 | 9,5 | 0,6 | 51,5 | 7,1 |
| NaOH55 | 8,7 | - | 9,9 | - | 48,1 | - |
| NaOH170 | 10,2 | 0,7 | 10,2 | 0,2 | 47,0 | 0,7 |
| CaO21 | 9,5 | - | 9,7 | - | 35,4 | 0,7 |
| CaOH55 | 8,6 | 0,2 | 9,4 | 0,6 | 37,8 | 0,1 |
| CaOH135 | 9,0 | 0,3 | 8,9 | 0,1 | 44,4 | - |

The percentage reduction of cellulose, hemicelluloses and lignin are presented in Figures 21 and 22.

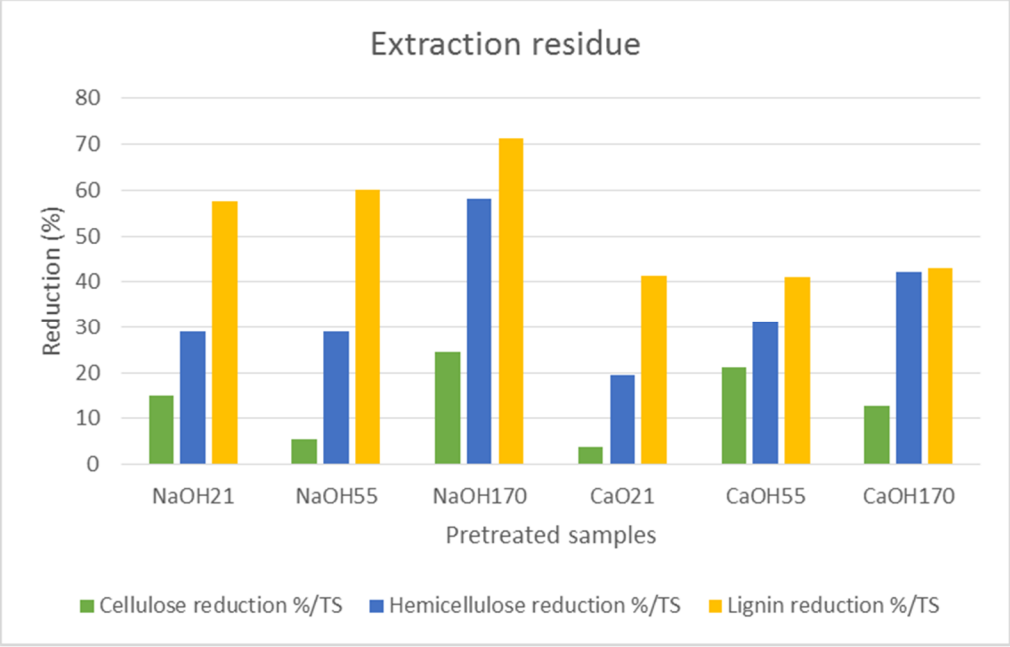



Figure 21 Reduction of cellulose, hemicellulose and lignin in pre-treated extraction residue sample compared to TS solubilisation

The effects of different pre-treatments in extraction residue samples are clear. The percentage amount of lignin has decreased in every sample, which leads to the conclusion that lignin was degraded and the aim of the pre-treatments was achieved. Lignin decreased at least 40 % in every sample. NaOH treatment seems to be more effective to dissolve lignin and the high temperature treatment has a positive reaction to the reaction. Lignin reduction is highest with NaOH at 170 °C but the results are directional,

because all dry matter wasn't collected after the pre-treatment (1 L autoclave). The amount of hemicellulose decreased as well, so it is possible to assume that it has solubilised.

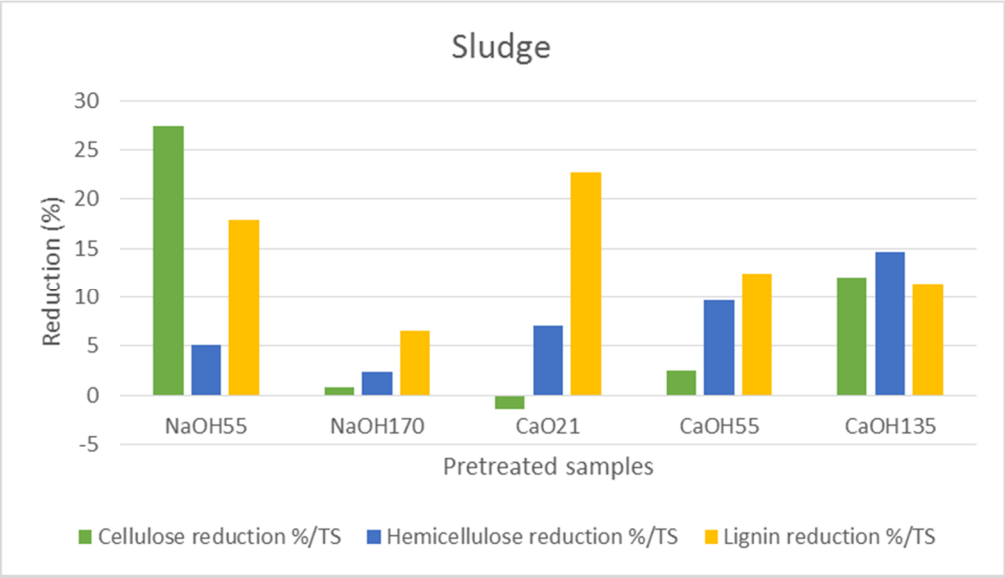



Figure 22 Reduction of cellulose, hemicellulose and lignin in pre-treated sludge sample compared to TS solubilisation

In the sludge sample, there are more variation between the results. Lignin hasn't dissolved as much as with the extraction residue sample. The highest solubilisation is in sample CaO at a temperature of 21 °C, where lignin has decreased 23 %. It seems that the high temperature treatment didn't have a positive influence. It can be assumed that pre-treatments have solubilised compounds to the liquid fraction because nearly all compounds decreased.

## 5.4 Characterisation of liquid after pre-treatment

### 5.4.1 COD

COD was determined from liquid fraction of the pre-treated samples. With the amount of COD it is also possible to calculate the theoretical amount of methane production where 1 g COD produces 350 ml methane, if all solubilized COD is fully converted into methane:

$$gCOD/L \times volume\ of\ liquid \times 350 ml CH_4/liquid\ fraction$$

The amount of COD and theoretical methane production from liquid fraction of the pre-treated samples are presented in Tables 11 and 12.

Table 11 Amount of COD and theoretical methane production from extraction residue sample

| Sample | gCOD/l | σ gCOD/l | mlCH4 from liquid fraction/ g initial TS |
|---|---|---|---|
| NaOH21 | 11,8 | 0,1 | 82,3 |
| NaOH55 | 12,0 | 0,1 | 83,8 |
| NaOH170 | 27,6 | 0,4 | 193,1 |
| CaO21 | 3,5 | 0,0 | 24,5 |
| CaOH55 | 3,7 | 0,0 | 26,1 |
| CaOH170 | 10,2 | 0,1 | 71,6 |

Table 12 Amount of COD and theoretical methane production from sludge sample

| Sample | gCOD/l | σ gCOD/l | mlCH4 from liquid fraction/ g initial TS |
|---|---|---|---|
| NaOH21 | 24,3 | 0,5 | 170,0 |
| NaOH55 | 22,9 | 0,1 | 160,0 |
| NaOH135 | 27,9 | 0,0 | 195,1 |
| CaO21 | 4,1 | 0,1 | 28,9 |
| CaOH55 | 4,2 | 0,4 | 29,2 |
| CaOH135 | 10,7 | 0,3 | 75,1 |

COD results show that NaOH pre-treatment is more efficient than lime pre-treatment to dissolve material to liquid fraction with both samples. A higher treatment temperature is more effective than a lower one. Sludge samples seem to be more solubilized than the extraction residue. It would have been good to find out, how much compounds could be solubilised to an aqueous phase (without pre-treatment), but this comparison was not performed.

### 5.4.2 Phenols

The amounts of released phenols are presented in Figures 23 and 24. They show that pre-treatments have contributed to break down lignin polymers or its bonds with holocelluloses. NaOH pre-treatment with a high temperature has a better effect compared to the other pre-treatments. Lime pre-treatments, however, have very low release of phenols from extraction residues and almost no phenol released from sludge samples.

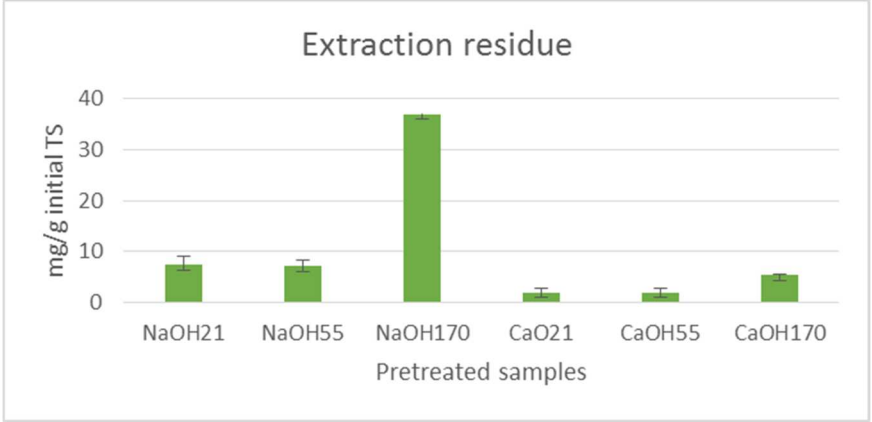


Figure 23 Amount of released phenols expressed mg/g initial TS of extraction residue sample

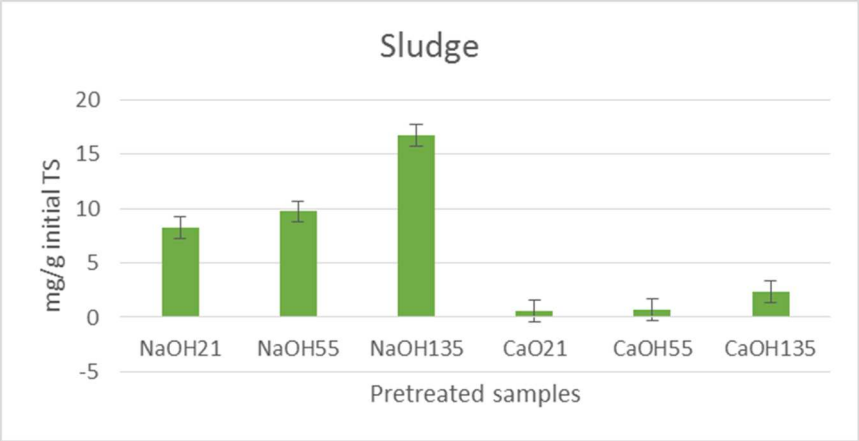



Figure 24 Amount of released phenols expressed mg/g initial TS of sludge sample

## 5.5 Results from BMP

The results from BMP tests are presented in Figures 25 and 27. The first test measured biogas production from the original bark and extraction residue and from the pre-treated extraction residue samples. The results are presented as ml/g TS.

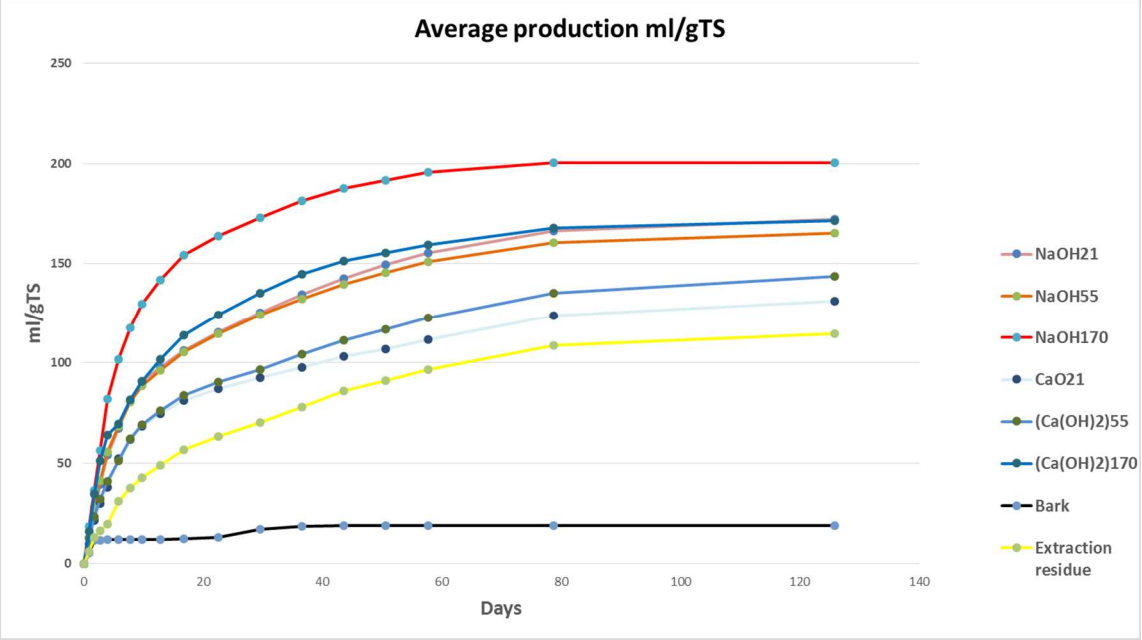



Figure 25 Biogas production with extraction residue, bark and pre-treated extraction residue samples

It is possible to see that original bark without treatment produces biogas poorly. All pre-treatments enhance gas production, but the most efficient

pre-treatment is NaOH pre-treatment at a high temperature. Other NaOH pre-treatments and $Ca(OH)_2$ pre-treatment at a high temperature have almost the same effect. Extraction residue without pre-treatment produces gas significantly if it is compared to the original bark. The comparison between the pre-treated and original extraction residue samples are presented in Figure 26. The results are presented as ml/gTS. It is possible to see that the high temperature pretreatment increases the methane production almost 80 %.

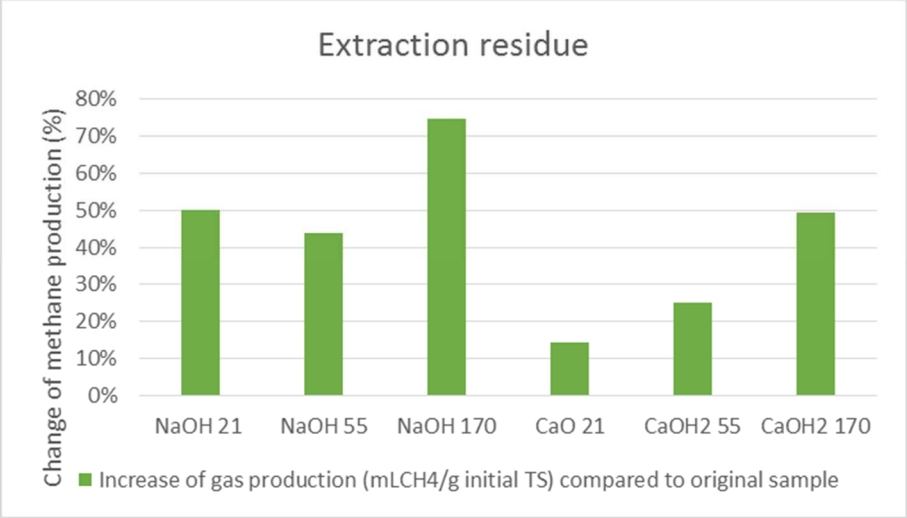



Figure 26 Increase of methane production compared to original extraction residue sample in BMP-test

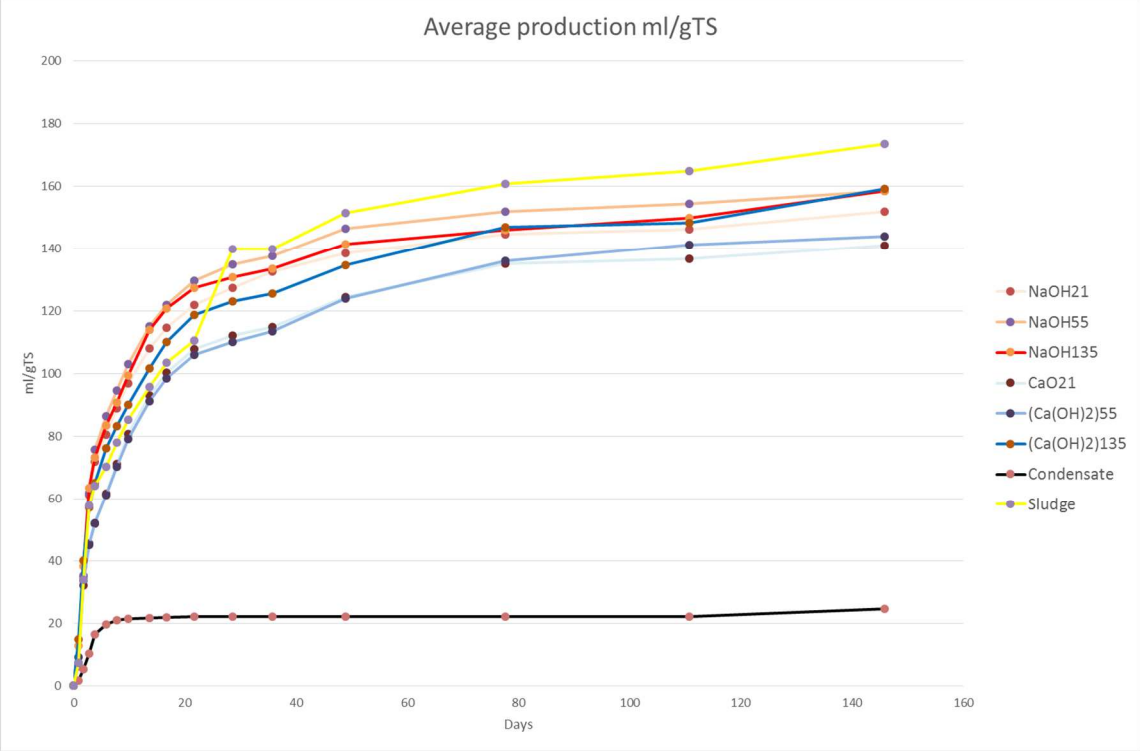



Figure 27 Biogas production with sludge, pyroligneous liquor and pretreated sludge samples

The results from the second BMP test are presented in Figure 27. Pyroligneous liquor produces biogas poorly (shown as condensate). The effects between different pre-treatments are slight. At the end of the test the original sludge sample produces more gas than the pre-treated ones. The comparison between the pre-treated and sludge samples are presented in Figure 28. The results are presented as ml/gTS. It is possible to see that the sample without pre-treatment produces more gas than with pretreatments.

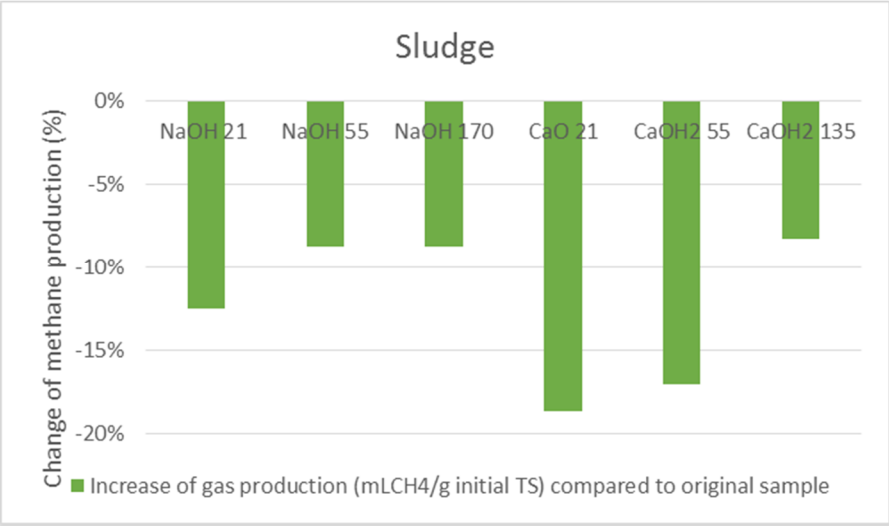



Figure 28 Change of methane production between original and pre-treated sludge samples in BMP-test

## 5.6 Results from Flash-BMP

The results from Flash-BMP are presented in Table 13. The results are from the original freeze dried samples and pre-treated samples. Flash-BMP was measured from the solid fraction of samples.

Table 13 Methane production estimated by Flash-BMP

| Sample | mlCH4/gVS | σ mlCH4/gVS |
|---|---|---|
| Bark | 84 | 4,6 |
| Extraction residue | 136 | 1,0 |
| Sludge | 122 | 10,5 |
| | | |
| Extraction residue | mlCH4/gVS | σ mlCH4/gVS |
| NaOH21 | 225 | 4,5 |
| NaOH55 | 221 | 0,0 |
| NaOH170 | 233 | 2,5 |
| CaO21 | 182 | 0,6 |
| CaOH55 | 151 | 6,3 |
| CaOH170 | 165 | 6,3 |
| | | |
| Sludge | mlCH4/gVS | σ mlCH4/gVS |
| NaOH21 | 176 | - |
| NaOH55 | 185 | - |
| NaOH135 | 163 | - |
| CaO21 | 174 | - |
| CaOH55 | 171 | - |
| CaOH135 | 161 | - |

Flash-BMP results and the results from the original samples do not vary significantly. Bark is still the most low-yielding sample of the original samples. In both tests, the pre-treated samples produce more biogas than the original samples. NaOH treatment for the extraction residue is more effective than the lime treatment, but the temperature does not cause clear differences between treatments. With sludge samples there is no large divergence between the pre-treatments, but all of them are more effective compared to the original sludge sample.

# 6 DISCUSSION

## 6.1 TS solubilisation and COD

Coincidence between TS solubilisation and COD results is to be seen. For extraction residue samples, the most effective pre-treatment has been NaOH treatment at a temperature of 170 °C. Comparing soda and lime pre-treatments, soda has been more effective in every case. High solubilisation with sludge samples in COD results can be explained with the texture of the sample. It was powdery and there was more surface for chemicals. An effective

solubilisation with NaOH treatment was proven by the colour of the pre-treated samples. Liquid fractions with NaOH pre-treatments were much darker than with lime pre-treatments with both samples. The amount of COD was higher with the soda treatment, so the chemical dissolved organic compounds from solid fraction to liquid fraction.

## 6.2 Lignin reduction and phenols

These results show that for the extraction residue, NaOH treatment at 170 °C is the most efficient pre-treatment for solubilize lignin, because amount of phenols is representing solubilisation of lignin. When phenols are solubilised to liquid fraction, it is performed also as in COD. In COD it is possible to see all organic matter, which are solubilised to liquid fraction. In both measurements with extraction residue, NaOH pre-treatment at a high temperature was the most efficient to dissolve lignin. The pre-treatment with NaOH was also the most effective to treat sludge samples. Calculation of biogas potential from COD of sludge samples is promising but BMP results show the opposite; pretreatments in sludge samples didn't have a positive impact on the methane production. High COD in sludge samples may be relatively high because of the small amount of solids in liquid fraction caused by the challenging separation between liquid and solid fraction.

## 6.3 Comparison of biogas production results

Biogas production measurements from BMP and Flash BMP and theoretical results from COD are shown in Tables 14 and 15. The results are presented in $mLCH_4$/g initial TS and $mLCH_4$/g initial VS to see the difference between TS and VS results. The results from BMP-test are the most reliable, because they are tested empirically.

Results from Flash BMP are from solid fraction so it is possible to compare theoretical results from solid fraction (result from BMP minus theoretical result from COD) to Flash BMP results. With these results it is possible to make conclusion that liquid fraction hasn't biodegradable totally, because amount of gas production in theoretical calculations are less than in Flash BMP.

With sludge samples difference between theoretical and Flash BMP results are negative in NaOH treatment, because theoretical results from liquid fraction are even higher than results from BMP, which are from both fractions. As we have seen, lignin has solubilised, but it seems that methane is not produced from degradation products of lignin.

Table 14 Comparison between different biogas measurement with extraction residue samples

| Sample | Results from BMP mLCH4/g initial TS | Results from BMP mLCH4/g initial VS | Theoritical mlCH4 from liquid fraction/g initial TS | Theoritical mlCH4 from liquid fraction/g initial VS | Theoritical mlCH4 from solid fraction/g initial TS | Theoritical mlCH4 from solid fraction/g initial VS | Results from flashBMP mlCH4/g initial VS |
|---|---|---|---|---|---|---|---|
| Extraction residue | 115 | **123** | | | | | **136** |
| NaOH 21 | 172 | **205** | 82 | **98** | 90 | **107** | **196** |
| NaOH 55 | 165 | **192** | 84 | **97** | 81 | **94** | **197** |
| NaOH 170 | 201 | **229** | 193 | **220** | 7 | **8** | **214** |
| CaO 21 | 131 | **162** | 25 | **30** | 106 | **132** | **149** |
| CaOH2 55 | 144 | **171** | 26 | **31** | 117 | **140** | **129** |
| CaOH2 170 | 171 | **207** | 72 | **86** | 100 | **121** | **138** |

Table 15 Comparison between different biogas measurement with sludge samples

| Sample | Results from BMP mLCH4/g initial TS | Results from BMP mLCH4/g initial VS | Theoritical mlCH4 from liquid fraction/g initial TS | Theoritical mlCH4 from liquid fraction/g initial VS | Theoritical mlCH4 from solid fraction/g initial TS | Theoritical mlCH4 from solid fraction/g initial VS | Results from flashBMP mlCH4/g initial VS |
|---|---|---|---|---|---|---|---|
| Sludge | 173 | **188** | | | | | **122** |
| NaOH21 | 152 | **177** | 170 | **199** | -18 | **-21** | **155** |
| NaOH55 | 158 | **184** | 160 | **186** | -2 | **-2** | **168** |
| NaOH135 | 158 | **201** | 195 | **248** | -37 | **-47** | **137** |
| CaO21 | 141 | **174** | 29 | **36** | 112 | **138** | **146** |
| CaOH55 | 144 | **175** | 29 | **36** | 115 | **140** | **144** |
| CaOH135 | 159 | **198** | 75 | **93** | 84 | **104** | **137** |

These results are comparable to other lignocellulosic substrates like wheat grass, rice straw, willow, miscanthus, paper tube residue and grass hay, which are producing approximately 160-230 l $CH_4$/kg VS. (Monlau, Barakat, Trably, Dumas, Steyer & Carrere. 2013)

TS of extraction residue was 44,3 % and the amount of VS was 94 % from TS. In general, methane production per ton of original raw material is presented in Table 16.

Table 16 Methane potential per ton of residue materials of SETA

| | Methane potential of raw material ($m^3$ $CH_4$/ton of VS) | VS amount /ton of raw material (kg) | Methane production (m3 methane/ton of raw material) |
|---|---|---|---|
| Extraction residue | 122,6 | 416,4 | 51,1 |
| Extraction residue with pretreatment (21°C + NaOH) | 204,6 | 416,4 | 85,2 |
| Sludge | 188,1 | 159,3 | 30,0 |

1 $m^3$ methane equals to 10 kWh energy, which can be compared to about 1 litre fuel oil. In general, with one ton of extraction residue it is possible to produce 51,1 $m^3$ methane, which is around 511 kWh. With a pre-treatment it is possible to increase methane production of extraction residue. Though, the potential of methane production from sludge is smaller compared to the extraction residue, because the VS content of sludge is smaller than with the extraction residues (initial samples).

# 7 CONCLUSIONS

As shown, original bark without treatments is not suitable for biogas production, because biogas production was very low. It seems that processing (extraction) of bark in Seta enhanced the biogas production and extraction residue without pre-treatment is already suitable for biogas production. However, it is observed that pre-treatments can enhance methane production significantly with extraction residue samples.

With extraction residue samples NaOH pre-treatments enhanced the biogas production significantly (60-80 % compared to the initial extraction residue samples). A high temperature treatment with NaOH was the most efficient pre-treatment. Other temperatures with NaOH and a high temperature pre-treatment with $CaOH_2$ were also effective to enhance methane production.

With sludge samples pre-treatments didn't have the expected influence on methane production. At the end of the test gas production was higher with the original sludge sample than the pre-treated ones. With analyses indicating the solubilisation of lignin, it was possible to forecast better biogas production. It seems that lignin has solubilised, but it does not produce methane in liquid fraction. There is a possibility to inhibition, if the treated samples from the tannin extraction process contained recalcitrant compounds or inhibitors.

Even though the pre-treatments had a more positive effect with extraction samples, it needs to be observed that without pre-treatments sludge produces better biogas than extraction residue (per VS). Pyroligneous liquor is not suitable for biogas production alone but could be mixed with the solid samples.

For industry, pre-treatments may be challenge to operate, because high treatment temperatures are expensive to reach. Positive information is that pre-treatment at a room temperature enhances methane production significantly. For example, if pre-treatment is operated at a room temperature for 5 days, it is easy for the chemical to mix to substrate before anaerobic digestion and storage of 5 days in tanks. However, if pre-treatments are operated, it will create costs from the chemicals and facilities and demand of space. It may also create some environmental questions.

In Seta the extraction residue is already utilized mainly in the process, so the major interest is to utilize sludge residue. With these results it is possible to draw a conclusion that sludge without pre-treatments produces biogas, but its organic matter content is quite low. However, it is possible to utilize it in biogas production and it doesn't need any chemicals to be added. It is also possible to mix it with other substrates.

# SOURCES


Ackmez, M. 2012. Biogas production: Pre-treatment Methods in Anaerobic Digestion. John Wiley & Sons.

Brown, AG & Chin Ko, H. 1997. Black Wattle and its Utilisation. Rural Industries Research & Development Corporation. Publication No. 97/77

Frigon, J-C & Guiot, S. Biomethane production from starch and lignocellulosic crops: a comparative review. 2010. Wiley InterScience.

Froehlich, P. & Moura, A. 2014. CARVÃO VEGETAL: PROPRIEDADES FÍSICO-QUÍMICAS E PRINCIPAIS APLICAÇÕES. Tecnologia e Tendências.

Furtado, C., dos Santos Stolz, A. Pinto, F. Moura, A., Dal Pont Morisso, F. Pitarelo, A.P., Ramos, L.P, von Mühlen, C. & Riegel-Vidotti, I. 2015. Pyroligneous liquir produced from *Acacia meansii* De Wild wood under controlled conditions as renewable source of chemicals. Quim. Nova, Vol. XY, No. 00, 1-7, 2015

Glazer, AW., Nikaido, H. 1995. Microbial biotechnology: fundamentals of applied microbiology. Freeman WH (ed) San Francisco, p 340. ISBN 0-71672608-4.

Gupta, Vijai Kumar, Tuohy, Maria G. Biofuel Technologies: Recent Developments. 2013. Springer.

Harmsen PFH, Huijen WJJ, Bermudez LLM, Bakker RRC. 2010. Literature review of physical and chemical pretreatment processes for lignocellulosic biomass. Food and Biobased Research, Wageningen UR, P 1-49.

Manya, J. 2012. Pyrolysis for Biochar Purposes: A Rewiew to Establish Current Knowledge Gaps and Research Needs. Environmental Science & Technology.

Menezes, C.M., Marder, L. Ben da Costa, A. 2013. Utilização da NIRS para a determinação de tanino e de umidade diretamente na casca de Acacia mearnsii para a indústria de extração. Universidade de Santa Cruz do Sul (UNISC), Santa Cruz do Sul. Brasil.

Monlau, F., Barakat, A., Trably, E., Dumas, C., Steyer, J-P., Carrere, H. Lignocellulosic Materials Into Biohydrogen and Biomethane: Impact of Structural Features and Pretreatment. 2013. Environmental Science and Technology.

Morais, E. 16.8.2015. Process Engineer. Seta. Email.

Mosier, N., Wyman, C., Dale, B., Elander, R., Lee, Y. Y., Holtzappe, M. & Ladisch, M. 2004. Features of promising technologies for pretreatment of lignocellulosic biomass. Bioresource Technology.

Mustafa, B., Mehmet, B., Elif, K., Havva, B. 2009. Main routes for the thermos-conversion of biomass into fuels and chemicals. Energy Convers Manage 50(12):3147-3157.

Lehmann, J. 2015. Biochar for Environmental Management: Science, Technology and Implementation (2nd Edition). Taylor and Francis.

Lesteur, M., Latrille, E., Bellon Maurel, V., Roger, J.M., Gonzales, C., Jonqua, G., Steyer, J.P. 2011. First step towards a fast analytical method for the determination of Biochemical Methane Potential of solid wastes by near infrared spectroscopy. Bioresource Technology. 2280-2288.

Osburn, L. & Osburn, J. 1993. Biomass Resources for Energy and Industry. http://www.ratical.org/renewables/biomass.html

Renner, R. 2014. Desenvolvimento de um processo de clareamento de taninos utilizando dioxido de enxofre ($SO_2$) como alvejante. Universidade de Santa Cruz do Sul (UNISC), Santa Cruz do Sul. Brasil.

Sambusii, C., Monlau, F., Ficara, E., Carrère, H., Malpei, F. 2013. A comparison of different pre-treatments to increase methane production from two agricultural substrates. Applied Energy.

Schwertner Charão, L. 2005. Polinização em *Acacia mearnsii* De Wild. Revista de Ciências Agro-Ambientais.

Seta. 2015. Company presentation.

Sluiter, A., Hames, B., Ruiz, R., Scarlata, C., Sluiter, J., Templeton, D., Croker, D. 2011. Laboratory Analytical Procedure (LAP). Determination of Structural Carbohydrates and Lignin in Biomass. National Renewable Energy Laboratory.

Sluiter, A., Ruiz, R., Scarlata, C,. Sluiter, J., Templeton, D. 2005. Laboratory Analytical Procedure (LAP). Determination of Extractives in Biomass. National Renewable Energy Laboratory.

Wall, J., Harwood, C. & Demain, A. Bioenergy. 2008. ASM Press.

Wertz, J-L & Bedue, O. Lignocellulosic Biorefineries. 2013. CRC Press.

Wessa. 2010. *Acacia mearnsii*. Accessed 21.10.2015 http://www.sciag.ukzn.ac.za/bcs/Photos%20weeds.htm

Zeki Berk. 2013. Freeze Drying (Lyophilization) and Freeze Concentration. Food Process Engineering and Technology (Second Edition). Food Science and Technology. 567-581.

| | | | | Pretreatment | | | | | BMP test | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Sample | Alkali | Pretreatment temperature | Bottle | Sample (g) | Alkali (g) | Water to pretreatment (g) | pH before pt | pH after pt | Macroelements (ml) | Oligoelements (ml) | Buffer solution | Sludge (ml) | Water for BMP (ml) |
| Extraction | NaOH | 21 | 1 | 2,0882 | 0,2045 | 40,03 | 12,73 | 10,74 | 8,6 | 4,0 | 20,8 | 44,4 | 282,14 |
| Extraction | NaOH | 21 | 2 | 2,077 | 0,2047 | 40,07 | 12,82 | 10,22 | 8,6 | 4,0 | 20,8 | 44,4 | 282,14 |
| Extraction | NaOH | 21 | 3 | 2,0741 | 0,2346 | 40 | 12,87 | 11,34 | 8,6 | 4,0 | 20,8 | 44,4 | 282,14 |
| Extraction | NaOH | 21 | 4 | 2,0814 | 0,2028 | 40,02 | 12,85 | 10,73 | | | | | |
| Extraction | NaOH | 45 | 5 | 2,0788 | 0,1982 | 40,03 | 12,71 | 10,3 | 8,6 | 4,0 | 20,8 | 44,4 | 282,14 |
| Extraction | NaOH | 45 | 6 | 2,0593 | 0,1974 | 40,01 | 12,78 | 10,03 | 8,6 | 4,0 | 20,8 | 44,4 | 282,14 |
| Extraction | NaOH | 45 | 7 | 2,0733 | 0,2171 | 40,02 | 12,8 | 10,5 | 8,6 | 4,0 | 20,8 | 44,4 | 282,14 |
| Extraction | NaOH | 45 | 8 | 2,0784 | 0,1961 | 40,05 | 12,77 | 10,16 | | | | | |
| Extraction | NaOH | 170 | 9 | 6,81 | | 35,4 | | 8,66 | 8,6 | 4,0 | 20,8 | 44,4 | 282,14 |
| Extraction | NaOH | 170 | 10 | 6,81 | | 35,41 | | 8,66 | 8,6 | 4,0 | 20,8 | 44,4 | 282,14 |
| Extraction | NaOH | 170 | 11 | 6,83 | | 35,41 | | 8,66 | 8,6 | 4,0 | 20,8 | 44,4 | 282,14 |
| Extraction | NaOH | 170 | 12 | 6,81 | | 35,41 | | 8,66 | | | | | |
| Extraction | CaO | 21 | 13 | 2,078 | 0,2679 | 40,04 | 12,57 | 12,01 | 8,6 | 4,0 | 20,8 | 44,4 | 282,14 |
| Extraction | CaO | 21 | 14 | 2,0881 | 0,2643 | 40,03 | 12,58 | 12,09 | 8,6 | 4,0 | 20,8 | 44,4 | 282,14 |
| Extraction | CaO | 21 | 15 | 2,0859 | 0,2652 | 40,04 | 12,55 | 12,07 | 8,6 | 4,0 | 20,8 | 44,4 | 282,14 |
| Extraction | CaO | 21 | 16 | 2,0799 | 0,265 | 40,05 | 12,57 | 12,15 | | | | | |
| Extraction | CaOH2 | 45 | 17 | 2,0855 | 0,2643 | 40,13 | 12,47 | 11,63 | 8,6 | 4,0 | 20,8 | 44,4 | 282,14 |
| Extraction | CaOH2 | 45 | 18 | 2,0618 | 0,2646 | 40,02 | 12,48 | 11,31 | 8,6 | 4,0 | 20,8 | 44,4 | 282,14 |
| Extraction | CaOH2 | 45 | 19 | 2,0786 | 0,2645 | 40,01 | 12,48 | 11,24 | 8,6 | 4,0 | 20,8 | 44,4 | 282,14 |
| Extraction | CaOH2 | 45 | 20 | 2,0706 | 0,2651 | 40 | 12,5 | 11,58 | | | | | |
| Extraction | CaOH2 | 170 | 21 | 7,83 | | 34,64 | | 10,79 | 8,6 | 4,0 | 20,8 | 44,4 | 282,14 |
| Extraction | CaOH2 | 170 | 22 | 7,84 | | 34,37 | | 10,79 | 8,6 | 4,0 | 20,8 | 44,4 | 282,14 |
| Extraction | CaOH2 | 170 | 23 | 7,83 | | 34,38 | | 10,79 | 8,6 | 4,0 | 20,8 | 44,4 | 282,14 |
| Extraction | CaOH2 | 170 | 24 | 7,83 | | 34,36 | | 10,79 | 8,6 | 4,0 | 20,8 | 44,4 | 282,14 |
| Bark | | | B1 | 2,0625 | | | | | 8,6 | 4,0 | 20,8 | 44,4 | 322,14 |
| Bark | | | B2 | 2,0706 | | | | | 8,6 | 4,0 | 20,8 | 44,4 | 322,14 |
| Bark | | | B3 | 2,0813 | | | | | 8,6 | 4,0 | 20,8 | 44,4 | 322,14 |
| Blank | | | O1 | | | | | | 8,6 | 4,0 | 20,8 | 44,4 | 322,14 |
| Blank | | | O2 | | | | | | 8,6 | 4,0 | 20,8 | 44,4 | 322,14 |
| Blank | | | O3 | | | | | | 8,6 | 4,0 | 20,8 | 44,4 | 322,14 |
| Extraction residue | | | R1 | 2,0642 | | | | | 8,6 | 4,0 | 20,8 | 44,4 | 322,14 |
| Extraction residue | | | R2 | 2,0658 | | | | | 8,6 | 4,0 | 20,8 | 44,4 | 322,14 |
| Extraction residue | | | R3 | 2,0632 | | | | | 8,6 | 4,0 | 20,8 | 44,4 | 322,14 |

Pretreatments and BMP test with sludge and pyroligneous liquor APPENDIX 1/2

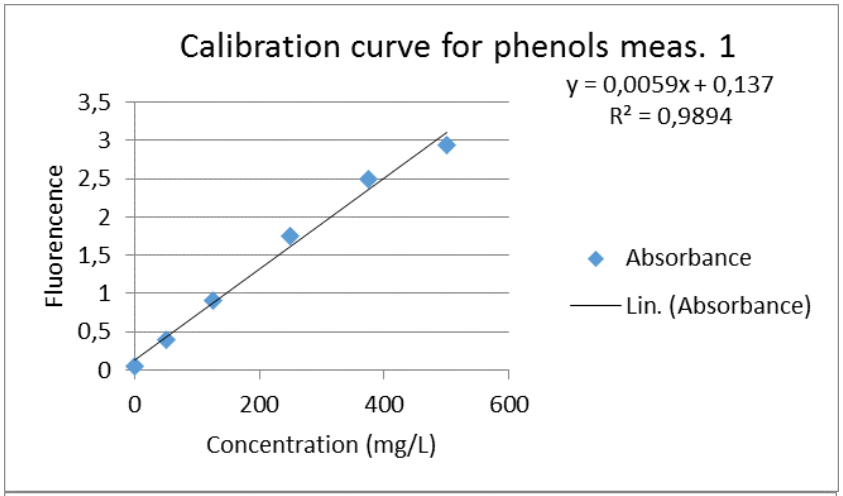
| | | | | Pretreatment | | | | | BMP test | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Sample | Alkali | Pretreatment temperature | Bottle | Sample (g) | Alkali (g) | Water to pretreatment (g) | pH before pt | pH after pt | Macroelements (ml) | Oligoelements (ml) | Buffer solution | Sludge (ml) | Water for BMP (ml) |
| Blank | | | 1 | | | | | | 8,6 | 4,0 | 20,8 | 44,4 | 322,14 |
| Blank | | | 2 | | | | | | 8,6 | 4,0 | 20,8 | 44,4 | 322,14 |
| Blank | | | 3 | | | | | | 8,6 | 4,0 | 20,8 | 44,4 | 322,14 |
| Sludge | NaOH | 21 | 4 | 2,0649 | 0,2084 | 40,02 | 11,9 | 8,1 | 8,6 | 4,0 | 20,8 | 44,4 | 282,14 |
| Sludge | NaOH | 21 | 5 | 2,0624 | 0,2017 | 40,07 | 11,77 | 8,16 | 8,6 | 4,0 | 20,8 | 44,4 | 282,14 |
| Sludge | NaOH | 21 | 6 | 2,0684 | 0,2135 | 40,01 | 11,86 | 8,08 | 8,6 | 4,0 | 20,8 | 44,4 | 282,14 |
| Sludge | NaOH | 21 | | 2,0659 | 0,23 | 40,02 | 12,05 | 7,98 | | | | | |
| Sludge | NaOH | 45 | 7 | 2,0632 | 0,202 | 40,03 | 11,53 | 9,15 | 8,6 | 4,0 | 20,8 | 44,4 | 282,14 |
| Sludge | NaOH | 45 | 8 | 2,0635 | 0,2028 | 40,04 | 11,73 | 9,22 | 8,6 | 4,0 | 20,8 | 44,4 | 282,14 |
| Sludge | NaOH | 45 | 9 | 2,0654 | 0,2205 | 40,04 | 11,79 | 9,41 | 8,6 | 4,0 | 20,8 | 44,4 | 282,14 |
| Sludge | NaOH | 45 | | 2,0661 | 0,197 | 40,02 | 11,6 | 9,1 | | | | | |
| Sludge | NaOH | 135 | 10 | 2,0665 | 0,2072 | 40 | 11,27 | 8,83 | 8,6 | 4,0 | 20,8 | 44,4 | 282,14 |
| Sludge | NaOH | 135 | 11 | 2,0646 | 0,1969 | 40,03 | 11,34 | 8,61 | 8,6 | 4,0 | 20,8 | 44,4 | 282,14 |
| Sludge | NaOH | 135 | 12 | 2,0606 | 0,2064 | 40 | 11,27 | 8,89 | 8,6 | 4,0 | 20,8 | 44,4 | 282,14 |
| Sludge | NaOH | 135 | | 2,0653 | 0,2053 | 40 | 11,42 | 8,9 | | | | | |
| Sludge | CaO | 21 | 13 | 2,0656 | 0,2071 | 40,03 | 11,93 | 9,03 | 8,6 | 4,0 | 20,8 | 44,4 | 282,14 |
| Sludge | CaO | 21 | 14 | 2,0662 | 0,201 | 40,03 | 12,01 | 9,03 | 8,6 | 4,0 | 20,8 | 44,4 | 282,14 |
| Sludge | CaO | 21 | 15 | 2,065 | 0,2011 | 40,02 | 12,23 | 9,02 | 8,6 | 4,0 | 20,8 | 44,4 | 282,14 |
| Sludge | CaO | 21 | | 2,0651 | 0,2044 | 40,03 | 12,22 | 9,09 | | | | | |
| Sludge | CaOH2 | 45 | 16 | 2,0659 | 0,2641 | 40 | 11,48 | 9,04 | 8,6 | 4,0 | 20,8 | 44,4 | 282,14 |
| Sludge | CaOH2 | 45 | 17 | 2,0674 | 0,2642 | 40 | 11,55 | 9,03 | 8,6 | 4,0 | 20,8 | 44,4 | 282,14 |
| Sludge | CaOH2 | 45 | 18 | 2,0655 | 0,2647 | 40,01 | 11,55 | 9,03 | 8,6 | 4,0 | 20,8 | 44,4 | 282,14 |
| Sludge | CaOH2 | 45 | | 2,0649 | 0,2645 | 40,03 | 11,63 | 9,05 | | | | | |
| Sludge | CaOH2 | 135 | 19 | 2,063 | 0,2649 | 40,03 | 11,07 | 7,99 | 8,6 | 4,0 | 20,8 | 44,4 | 282,14 |
| Sludge | CaOH2 | 135 | 20 | 2,0655 | 0,2642 | 40 | 11,19 | 8,01 | 8,6 | 4,0 | 20,8 | 44,4 | 282,14 |
| Sludge | CaOH2 | 135 | 21 | 2,0631 | 0,2645 | 40,02 | 11,1 | 8,01 | 8,6 | 4,0 | 20,8 | 44,4 | 282,14 |
| Sludge | CaOH2 | 135 | | 2,0619 | 0,2649 | 40,04 | 11,27 | 8,06 | | | | | |
| Condensate* | | | 22 | 10,0* | | | | | 4,3 | 4,0 | 20,8 | 44,4 | 316,45 |
| Condensate* | | | 23 | 10,0* | | | | | 4,3 | 4,0 | 20,8 | 44,4 | 316,45 |
| Condensate* | | | 24 | 10,0* | | | | | 4,3 | 4,0 | 20,8 | 44,4 | 316,45 |
| Sludge | | | 25 | 2,0615 | | | | | 8,6 | 4,0 | 20,8 | 44,4 | 322,14 |
| Sludge | | | 26 | 2,061 | | | | | 8,6 | 4,0 | 20,8 | 44,4 | 322,14 |
| Sludge | | | 27 | 2,068 | | | | | 8,6 | 4,0 | 20,8 | 44,4 | 322,14 |

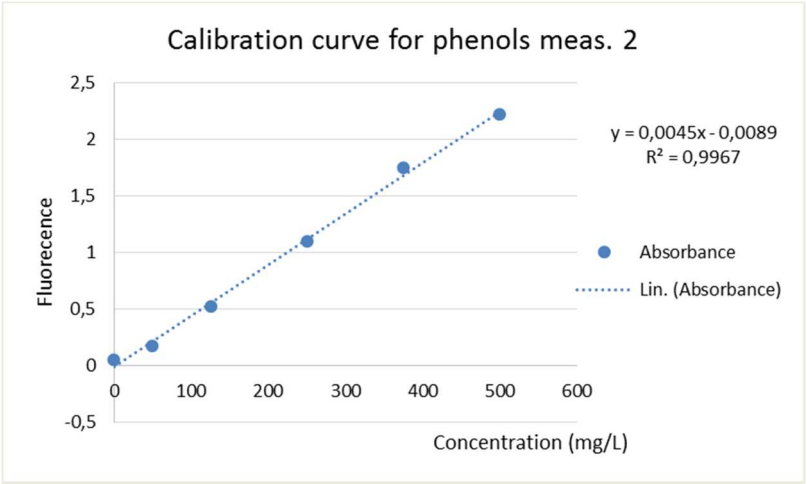
*=ml

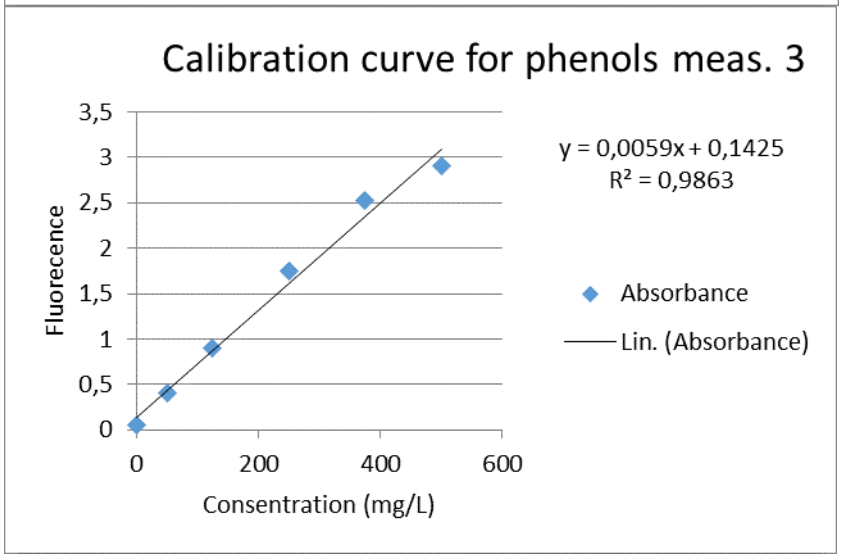
APPENDIX 2

Dry weight and solubilisation

| Extraction residue | | | | |
|---|---|---|---|---|
| Sample | Dry weight before pt (g) | Dry weight after pt (g) | Loss (g) | TS solubisation % |
| NaOH 21 | 2,0814 | 1,4943 | 0,5871 | 28,21 |
| NaOH 55 | 2,0784 | 1,577 | 0,5014 | 24,12 |
| NaOH 170 | 30,0602 | 14,99 | 15,0702 | 50,13 |
| CaO 21 | 2,0799 | 1,9156 | 0,1643 | 7,90 |
| CaOH2 55 | 2,0706 | 1,7445 | 0,3261 | 15,75 |
| CaOH2 170 | 30,9043 | 22,47 | 8,4343 | 27,29 |
| Sludge | | | | |
| Sample | Dry weight before pt (g) | Dry weight after pt (g) | Loss (g) | TS solubisation % |
| NaOH55 | 2,0661 | 1,447 | 0,6191 | 29,96 |
| NaOH170 | 2,0653 | 1,683 | 0,3823 | 18,51 |
| CaO21 | 2,0651 | 1,847 | 0,2181 | 10,56 |
| CaOH55 | 2,0649 | 1,96 | 0,1049 | 5,08 |
| CaOH135 | 2,0619 | 1,687 | 0,3749 | 18,18 |


Calibration curve for phenols meas. 1
y = 0,0059x + 0,137
R² = 0,9894
Fluorencence
3,5
3
2,5
2
1,5
1
0,5
0
0
200
400
600
Concentration (mg/L)
Absorbance
Lin. (Absorbance)


Calibration curve for phenols meas. 2
y = 0,0045x - 0,0089
R² = 0,9967
Fluorecence
2,5
2
1,5
1
0,5
0
-0,5
0
100
200
300
400
500
600
Concentration (mg/L)
Absorbance
Lin. (Absorbance)


Calibration curve for phenols meas. 3
y = 0,0059x + 0,1425
R² = 0,9863
Fluorecence
3,5
3
2,5
2
1,5
1
0,5
0
0
200
400
600
Consentration (mg/L)
Absorbance
Lin. (Absorbance)

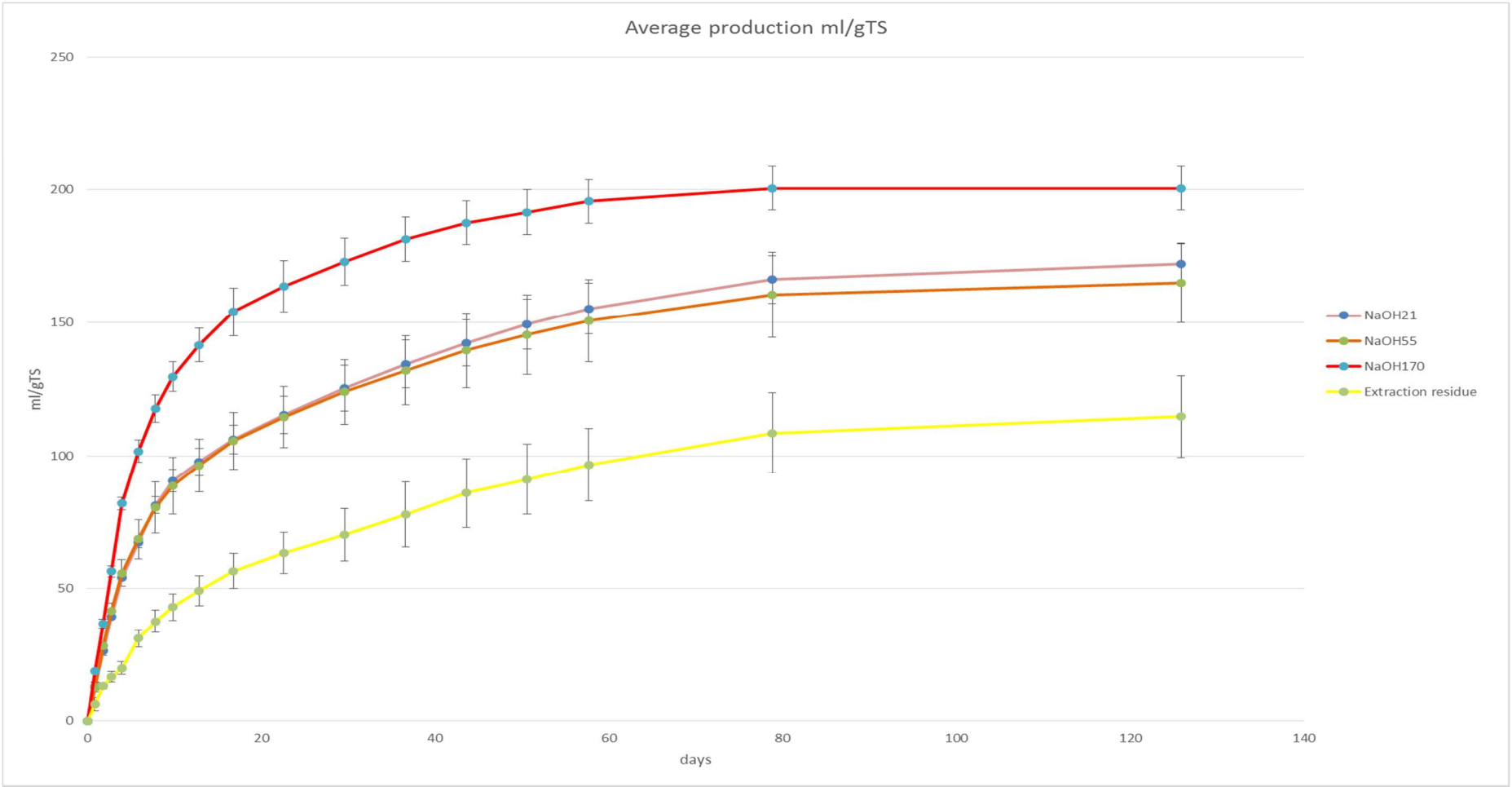
BMP results from extraction residue test

APPENDIX 4/1

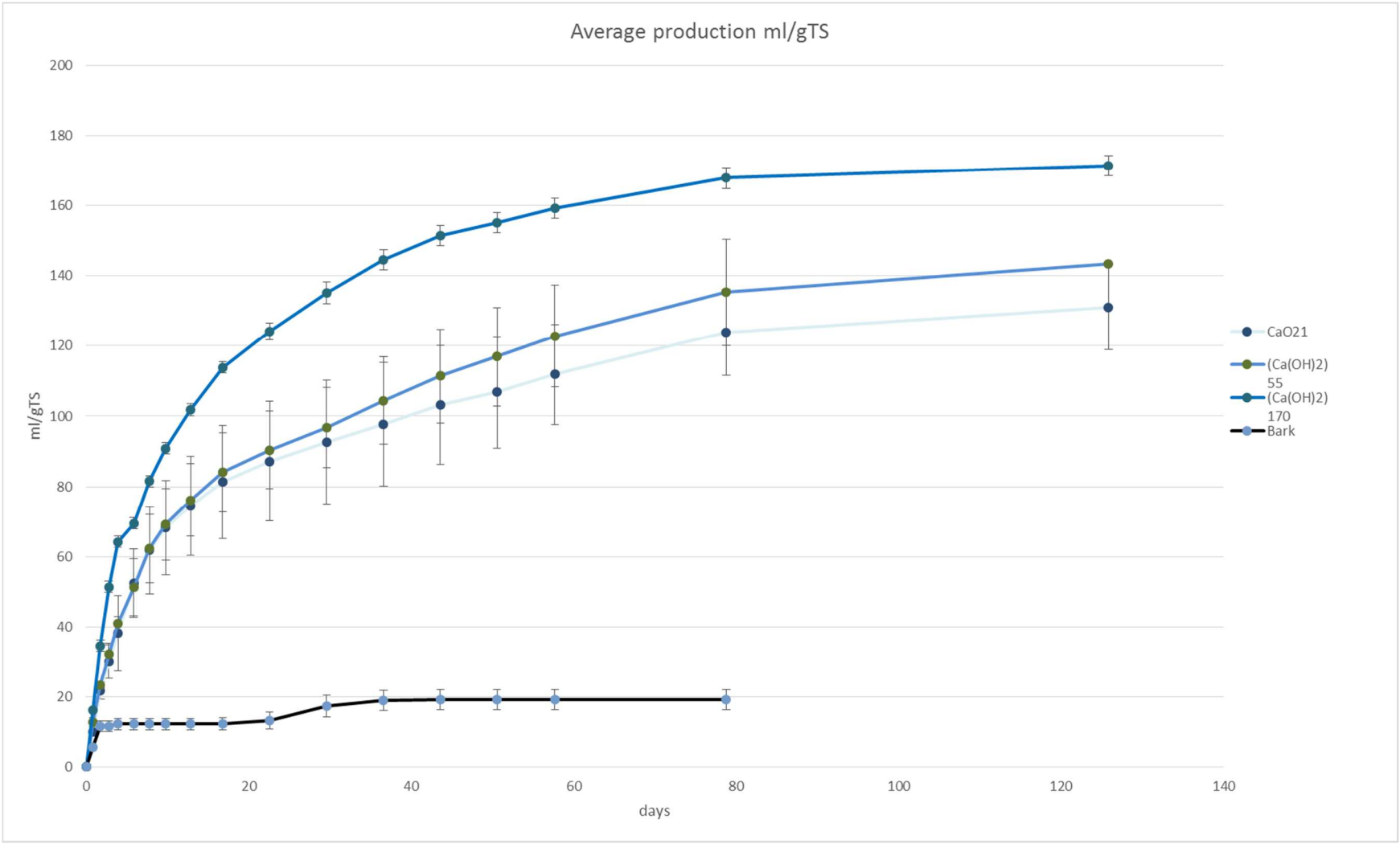
**Average production of triplicate ml/gTS**

| Measurement | days | NaOH21 | | NaOH55 | | NaOH170 | | CaO21 | | CaOH55 | | CaOH170 | | Bark | | Extraction residue | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Average | ndard deviat | Average | ndard deviat | Average | ndard deviat | Average | ndard deviat | Average | ndard deviat | Average | andard deviatio | Average | tandard deviatio | Average | andard deviatio |
| 0 | 0,00 | 0,0 | 0,0 | 0,0 | 0,0 | 0,0 | 0,0 | 0,0 | 0,0 | 0,0 | 0,0 | 0,0 | 0,0 | 0,0 | 0,0 | 0,0 | 0,0 |
| 1 | 0,85 | 12,9 | 1,4 | 12,7 | 1,8 | 18,6 | 0,7 | 10,0 | 1,3 | 12,7 | 1,2 | 16,2 | 0,4 | 5,6 | 0,4 | 6,2 | 2,6 |
| 2 | 1,77 | 26,3 | 1,7 | 28,1 | 1,4 | 36,7 | 1,8 | 21,7 | 2,4 | 23,3 | 0,9 | 34,6 | 1,6 | 11,5 | 1,6 | 13,1 | 1,2 |
| 3 | 2,77 | 39,4 | 0,1 | 41,4 | 3,0 | 56,3 | 2,2 | 30,1 | 4,8 | 32,2 | 3,0 | 51,4 | 1,6 | 11,5 | 1,6 | 16,6 | 1,9 |
| 4 | 3,94 | 54,0 | 0,2 | 55,7 | 5,0 | 82,0 | 2,3 | 38,2 | 10,7 | 41,0 | 1,9 | 64,2 | 1,6 | 12,2 | 1,6 | 19,8 | 2,4 |
| 5 | 5,85 | 67,2 | 1,9 | 68,5 | 7,5 | 101,8 | 4,3 | 52,4 | 9,8 | 51,4 | 8,1 | 69,5 | 1,6 | 12,2 | 1,6 | 31,0 | 3,2 |
| 6 | 7,81 | 81,4 | 3,1 | 80,4 | 9,6 | 117,7 | 5,2 | 61,8 | 12,4 | 62,3 | 9,8 | 81,6 | 1,6 | 12,2 | 1,6 | 37,5 | 4,1 |
| 7 | 9,81 | 90,6 | 4,1 | 88,6 | 10,6 | 129,7 | 5,6 | 68,3 | 13,4 | 69,2 | 10,2 | 90,9 | 1,6 | 12,2 | 1,6 | 42,9 | 5,0 |
| 8 | 12,85 | 97,6 | 5,1 | 96,3 | 9,9 | 141,6 | 6,3 | 74,6 | 14,1 | 76,2 | 10,3 | 101,8 | 1,6 | 12,2 | 1,6 | 49,1 | 5,7 |
| 9 | 16,81 | 106,2 | 5,4 | 105,6 | 10,8 | 154,1 | 8,9 | 81,3 | 16,2 | 84,0 | 11,3 | 113,9 | 1,6 | 12,2 | 1,6 | 56,6 | 6,6 |
| 10 | 22,58 | 115,4 | 7,0 | 114,7 | 11,5 | 163,7 | 9,7 | 87,2 | 17,1 | 90,4 | 11,1 | 124,1 | 2,3 | 13,2 | 2,3 | 63,3 | 7,8 |
| 11 | 29,59 | 125,4 | 8,6 | 124,0 | 12,1 | 172,9 | 8,8 | 92,6 | 17,6 | 96,8 | 11,4 | 135,1 | 3,1 | 17,2 | 3,1 | 70,2 | 9,8 |
| 12 | 36,61 | 134,5 | 9,0 | 132,1 | 13,0 | 181,4 | 8,4 | 97,7 | 17,6 | 104,5 | 12,4 | 144,7 | 2,9 | 18,8 | 2,9 | 77,9 | 12,3 |
| 13 | 43,61 | 142,4 | 8,7 | 139,6 | 14,0 | 187,5 | 8,2 | 103,2 | 16,9 | 111,4 | 13,4 | 151,4 | 2,9 | 19,0 | 2,9 | 86,0 | 12,9 |
| 14 | 50,58 | 149,4 | 9,3 | 145,5 | 14,9 | 191,5 | 8,5 | 106,8 | 15,9 | 117,0 | 14,0 | 155,2 | 2,9 | 19,1 | 2,9 | 91,2 | 13,2 |
| 15 | 57,69 | 155,4 | 9,5 | 150,7 | 15,6 | 195,5 | 8,3 | 111,9 | 14,2 | 122,8 | témoins froids | 159,3 | 2,9 | 19,1 | 2,9 | 96,6 | 13,7 |
| 16 | 78,80 | 166,3 | 8,9 | 160,5 | 15,9 | 200,5 | 8,3 | 123,9 | 12,2 | 135,3 | 15,2 | 167,9 | 2,9 | 19,1 | 2,9 | 108,6 | 15,0 |
| 17 | 125,8 | 172,2 | 7,5 | 165,1 | 14,9 | 200,5 | 8,3 | 131,0 | 12,1 | 143,5 | 15,3 | 171,4 | 2,9 | 19,1 | 2,9 | 114,8 | 15,3 |

BMP graphs from extraction residue test with standard deviation (NaOH-bottles and reference) APPENDIX 4/2



BMP graphs from extraction residue test with standard deviation (CaO- and $CaOH_2$-bottles and bark) APPENDIX 4/3



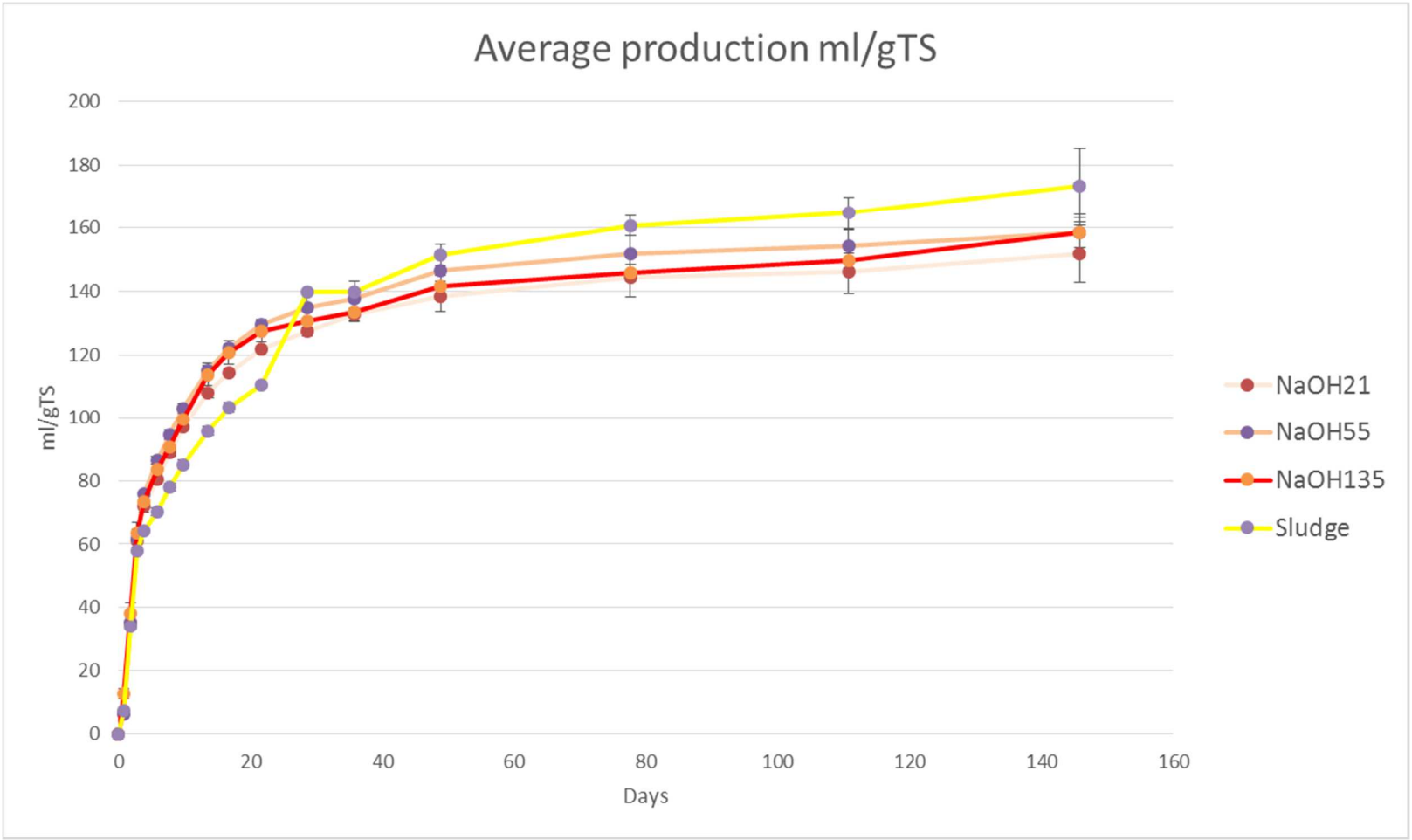
BMP results from sludge test

APPENDIX 4/4

**Average production of triplicate ml/gTS**

| Measurement | days | NaOH21 | | NaOH55 | | NaOH135 | | CaO21 | | CaOH55 | | CaOH135 | | Condensate | | Sludge | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Average | ndard deviat | Average | ndard deviat | Average | ndard deviat | Average | ndard deviat | Average | ndard deviat | Average | ndard deviat | Average | ndard deviat | Average | ndard deviat |
| 0 | 0,00 | 0,0 | 0,0 | 0,0 | 0,0 | 0,0 | 0,0 | 0,0 | 0,0 | 0,0 | 0,0 | 0,0 | 0,0 | 0,0 | 0,0 | 0,0 | 0,0 |
| 1 | 0,81 | 6,8 | 1,5 | 6,2 | 1,1 | 12,7 | 1,6 | 7,3 | 0,6 | 9,2 | 1,2 | 14,8 | 1,9 | 1,6 | 0,1 | 7,3 | 0,9 |
| 2 | 1,81 | 34,9 | 1,8 | 35,3 | 1,8 | 38,2 | 3,3 | 32,0 | 0,3 | 33,8 | 2,2 | 40,0 | 2,5 | 5,3 | 0,4 | 34,0 | 1,2 |
| 3 | 2,76 | 61,2 | 2,3 | 61,9 | 2,7 | 63,4 | 3,4 | 45,5 | 0,3 | 45,0 | 1,0 | 57,2 | 1,3 | 10,3 | 0,7 | 57,8 | 1,2 |
| 4 | 3,80 | 71,9 | 1,6 | 75,7 | 0,7 | 73,3 | 2,4 | 51,9 | 0,9 | 52,1 | 0,9 | 65,1 | 1,6 | 16,5 | 0,8 | 64,2 | 1,3 |
| 5 | 5,81 | 80,6 | 1,4 | 86,5 | 1,2 | 83,5 | 2,6 | 61,5 | 1,6 | 61,2 | 1,7 | 76,2 | 1,7 | 19,6 | 0,2 | 70,3 | 1,1 |
| 6 | 7,80 | 88,8 | 1,2 | 94,6 | 1,5 | 90,7 | 2,8 | 71,3 | 1,5 | 70,2 | 1,9 | 83,3 | 1,7 | 20,9 | 0,2 | 78,0 | 1,3 |
| 7 | 9,81 | 96,9 | 1,0 | 103,0 | 1,5 | 99,4 | 3,2 | 80,6 | 1,3 | 79,1 | 1,6 | 90,1 | 1,9 | 21,5 | 0,2 | 85,2 | 1,4 |
| 8 | 13,59 | 108,0 | 1,5 | 115,0 | 1,5 | 113,8 | 3,6 | 93,0 | 1,4 | 91,2 | 1,9 | 101,6 | 1,8 | 21,7 | 0,2 | 95,7 | 1,3 |
| 9 | 16,68 | 114,5 | 1,2 | 121,9 | 1,5 | 120,8 | 3,6 | 100,3 | 1,6 | 98,5 | 2,5 | 110,0 | 2,5 | 22,0 | 0,2 | 103,3 | 1,5 |
| 10 | 21,68 | 121,9 | 0,7 | 129,5 | 1,7 | 127,4 | 3,4 | 107,8 | 1,5 | 106,0 | 2,6 | 118,7 | 3,0 | 22,1 | 0,3 | 110,6 | 1,0 |
| 11 | 28,59 | 127,3 | 1,4 | 134,8 | 4,3 | 130,7 | 3,0 | 112,0 | 1,6 | 110,1 | 2,6 | 123,0 | 3,8 | 22,1 | 0,3 | 139,8 | 0,8 |
| 12 | 35,76 | 132,6 | 1,9 | 137,5 | 5,7 | 133,4 | 2,9 | 114,7 | 1,7 | 113,6 | 1,2 | 125,5 | 4,3 | 22,1 | 0,3 | 139,8 | 0,8 |
| 13 | 48,84 | 138,4 | 4,7 | 146,4 | 6,3 | 141,5 | 3,3 | 124,4 | 1,6 | 123,8 | 5,7 | 134,6 | 5,5 | 22,1 | 0,3 | 151,3 | 3,3 |
| 14 | 77,67 | 144,5 | 6,1 | 151,8 | 5,8 | 145,9 | 2,7 | 135,1 | 2,7 | 136,0 | 4,6 | 146,7 | 6,2 | 22,1 | 0,3 | 160,8 | 3,2 |
| 15 | 110,75 | 146,2 | 7,0 | 154,4 | 4,8 | 149,7 | 2,4 | 136,5 | 1,4 | 141,1 | temoins froids | 148,2 | 5,1 | 22,1 | 0,3 | 164,7 | 5,0 |
| 16 | 145,84 | 151,7 | 9,0 | 158,3 | 6,0 | 158,3 | 4,7 | 141,0 | 3,9 | 143,8 | temoins froids | 159,1 | 4,9 | 24,5 | 0,3 | 173,4 | 11,6 |

BMP graphs from sludge test with standard deviation (NaOH-bottles and reference) APPENDIX 4/5



BMP graphs from sludge test with standard deviation (CaO- and $CaOH_2$-bottles and pyroligneous liqour) APPENDIX 4/6